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BACTERIA IN RELATION TO MAN

LIPPINCOTT'S NURSING MANUALS

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Individual resistance in plants is illustrated by the behavior of these various varieties of tomatoes grown on wilt-infected soil. The varieties represented are: Row 1, Norton (selection from Stone); row 2, selection from Greater Baltimore; row 3, Louisiana Wilt Resistant; row 4, Earliana; row 5, Bonny Best; row 6, Duke of York; row 7, Early Detroit; row 8, Ponderosa; row 9, Greater Baltimore. (Pritchard, U. S. Dept. Agriculture.)

LIPPINCOTT'S NURSING MANUALS

BACTERIA IN RELATION TO MAN

A STUDY-TEXT IN GENERAL MICROBIOLOGY

BY

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147 ILLUSTRATIONS

PHILADELPHIA AND LONDON
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TO

AUSTIN C. APGAR

WHO GAVE TO THE ABSTRACT SCIENCES
OF HIS DAY RICH HUMAN RELATIONSHIPS

63345

PREFACE

Since this combined text and laboratory outline is designed as an introduction to microbiology, it begins with a general discussion of cells, including those of ordinary green plants. This background is indispensable, because all the helpful and harmful relations of micro-organisms depend upon their characteristics and activities as living cells—upon the materials and conditions essential for their growth and multiplication, and their ability to resist or meet changes in environmental factors.

This book should not replace the standard texts on bacteriology. The topical arrangement is designed to encourage its use as a skeleton or ground work to which the student may add enriching material gained from such text-books, as well as from contemporary reports and publications.

Much of the matter ordinarily covered as laboratory work is given here in demonstration form. This is highly desirable in a beginning course for general students, for technical skill is needed only by specialists in any given field. For all others breadth of content and general "appreciations" are much more important. These ends will be more surely attained if the instructor uses similar rather than identical material in the demonstration exercises, thus *adding* to the demonstration values.

The open spacing is designed to encourage notes of a thoughtful type, for it affords space for drawings or notes when their need is felt, and may incidentally discourage the rather common habit of voluminous, unselective note taking, so likely to be indulged in when notes are taken in a note-book and separated from the text to which they are related.

Part II of this book, How We Resist Disease, is bound as a separate volume. The first three chapters form a brief introduction to the complex subject of immunity. References to it in this first volume carry the Roman number II (*e.g.*, Fig. 58, II).

The limited amount of purely technical material in this course allows greater emphasis on the practical phases of bac-

teriology, and the student should profit by the valuable material to be found in the general hygiene references listed in the appendix with the standard text-books on bacteriology.

Particular attention is called to the final chapter. Interested students who have time for the suggested readings will find it most suggestive and stimulating.

JEAN BROADHURST

TEACHERS COLLEGE, COLUMBIA UNIVERSITY,
New York, October, 1924.

ACKNOWLEDGMENTS

SINCERE acknowledgment is made of the helpful criticism by former students who, during the past ten years, have used this outline in its preliminary form as a loose-leaf outline.

Grateful mention is also due the many publishers and scientific investigators who have generously granted permission for the reproduction of the illustrations reappearing here. Each courtesy is definitely credited in the descriptive legends.

Practically all of the original drawings were made by Mrs. Ivan G. Doubble, and the credit for most of the original photographs should be given to Mrs. Maurice Ricker.

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BACTERIA IN RELATION TO MAN

INTRODUCTORY WORK WITH THE COMPOUND MICROSCOPE

Preliminary Requirements.—Students who are not thoroughly at home with the microscope are expected to become familiar with its workings before the first week ends. A working knowledge means (1) skill in alternating the high and low powers, varying objectives, oculars, or both; and (2) the power to use intelligently (*a*) the coarse and fine adjustments, (*b*) the mirrors, and (*c*) the diaphragm. It, therefore, implies a sense of discrimination that tells when a given adjustment of lenses, mirror, and diaphragm is yielding the best illumination and the sharpest definition possible. Students who have not yet acquired the habit of focusing *up* (never down) while looking through the tube, or who do not know the approximate focal distance of the different objectives, still need much practice with the microscope. It is essential that the fingers should be "sensitive" to the fine adjustment screw, (1) telling instantly when it ceases to affect the focus, and (2) automatically limiting all movement of this screw to but a fraction of a turn. With the oil-immersion lenses it is absolutely essential that one hand should be kept in constant touch with the fine adjustment screw. All who find in the following review that they are not sufficiently familiar with the microscope are requested to arrange for extra practice *before* the next session of the class.

The microscopes required for ordinary work are *simple* and *compound*.

The Simple Microscope.—The simple microscope, used for studying "colonies" or other masses of bacterial growth, may be either an ordinary pocket lens or one of the more elaborate dissecting microscopes. Microscopes or magnifying glasses of this class range in magnification from two to forty diameters; that is, the diameter of the image seen

in the microscope is two to forty times the real diameter of the object itself. Usually, however, for magnifications higher than about twenty diameters, the compound microscope is used.

1. THE COMPOUND MICROSCOPE

The Microscope Stand.—The accompanying labelled picture (Fig. 1) will help in identifying the more important mechanical parts of the compound microscope. Locate the heavy foot, or *base,* often made in the shape of a horseshoe, and the upright *pillar,* which is sometimes supplied with a joint, or hinge, allowing inclination of the instrument. (For our work this hinge is rarely used.) The *stage* is the horizontal support or shelf-like part, upon which is placed the object to be examined (mounted, usually upon a glass slide). The stage is provided with a pair of metal clips; these are used to hold the glass slip, or object slide, in position.

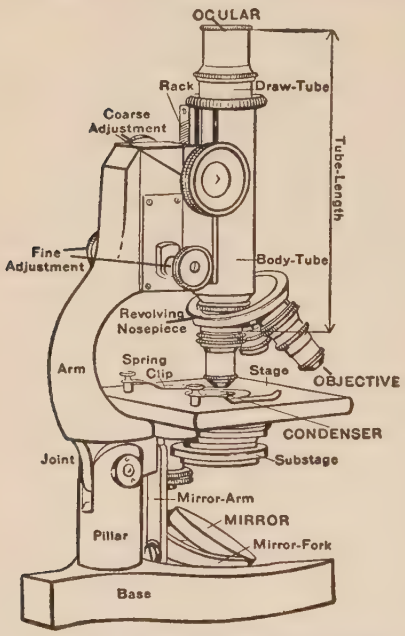

Fig. 1.—Identify on your microscope each of the parts named in this illustration. How many of these are immovable? Which are adjustable? Which adjustments affect the light conditions? Which are necessary to obtain a sharp or exact focus? (From Gage: The Microscope, Comstock.)

The *mirror* reflects the light through the hole in the stage, and thus illuminates the object slide. The mirror is so arranged that it may be turned in various directions and thus secure a maximum amount of light without shifting the position of the microscope itself. This enables the worker to maintain the same relative position with regard to the microscope, and to manage the necessary adjustments automatically, without taking his eye from the eyepiece.

Immediately beneath the stage, in some instruments, is a revolving plate with holes of varying sizes, called the *diaphragm;* this is used to regulate the amount of light passing from the mirror into the lenses. In other instruments cylindrical plugs with hollow cavities or apertures of different sizes are provided; these may be inserted into the hole in the stage, and serve the same purpose as the diaphragm described above. A third style of diaphragm is the iris diaphragm; examine microscopes until you find one of these iris diaphragms, made of sliding, overlapping metal strips. These are found only on the better microscopes. They are much more easily injured, but are often preferred as they allow very exact light adjustment.

The *arm* of the microscope reaching out from the pillar supports more or less directly the *tube,* which in turn carries within itself the *draw-tube.* The tube may be moved up and down by the rack-and-pinion screws—the *"coarse adjustment"*; more delicate motion is secured by the use of the *fine adjustment,* which is regulated by a small milled wheel, generally placed horizontally at the top of the pillar. In some of the newer microscopes, this fine adjustment is controlled by a *pair* of smaller wheels placed *vertically* just below the rack-and-pinion or coarse adjustment wheels. Find one of this type and use it within the next two or three lessons.

Careful work with high powers makes it necessary to readjust the focus constantly; experienced workers, therefore, keep the thumb and forefinger in play on the fine adjustment screw. Students should acquire this habit, as it eliminates much of the eye-strain felt by beginners, who otherwise strain the eyes by attempting to see objects not constantly or not sharply in focus. Another great advantage in this habit is that slight variations in focal distance give the worker a feeling of depth or third dimension with regard to the object studied, by bringing successively the upper, middle and lower regions or parts of the object into focus, thus compensating somewhat for the lack of perspective met in the ordinary microscope, where but one eye is used.

The Optical Parts.—The parts of the microscope thus far mentioned are generally included in the term *"microscope stand."* The optical parts consist of (1) low-power lenses: *eye-*

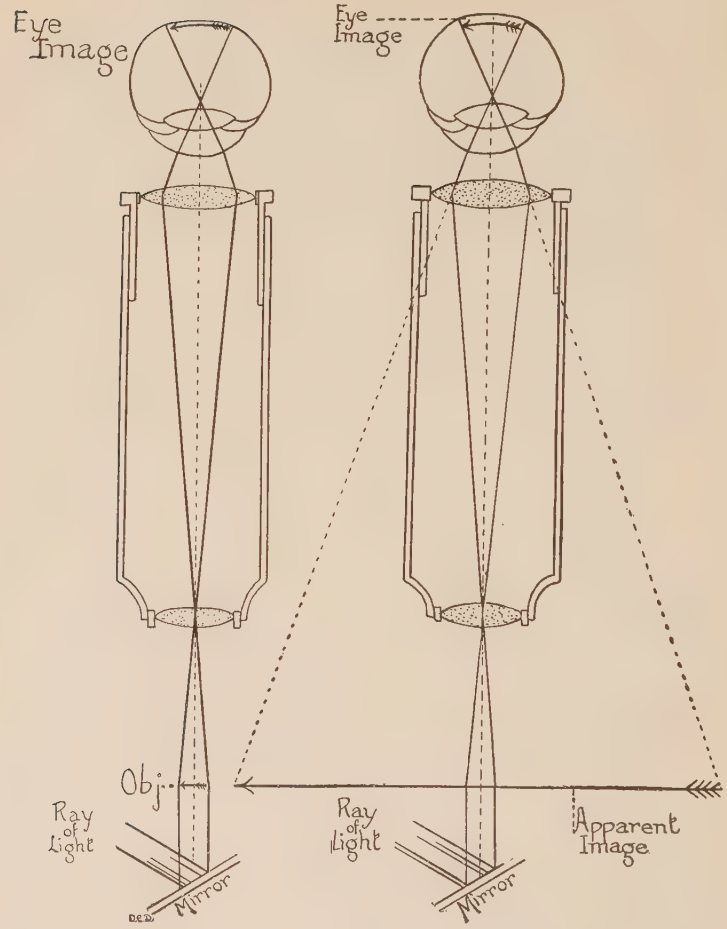

FIG. 2.—The left diagram shows part of the light rays passing from the object to be magnified (arrow) to the eye. Impressions on the retina are translated by us in terms of our past experience, both in perspective and distance. Objects with boundaries represented by the angle at the eye shown here, will be interpreted in sizes corresponding more or less to the distance of the object from the eye. Continuing the angles at the eye to the neighborhood of the arrow leads us to feel or "see" an enlarged arrow, as shown by the dotted line, in the right diagram.

pieces or *oculars,* which fit into the upper part of the draw-tube; and (2) lenses of still higher magnification: *objectives,* which screw into the lower end of the draw-tube, near the object to

be studied. A very convenient, additional piece of apparatus is the *nose-piece,* by which two or three objectives, revolving around a common center, may be attached to the lower end or "nose" of the tube, permitting an instantaneous change in the magnification of the object. Each ocular and objective is really a combination of lenses and not a single lens. Turn the ocular over in your hand and note that there is a lens at each end of the metal cylinder that holds the two together. It is, however,

Magnification Table

Tube length, 160 mm. **Image distance, 250 mm.**

Objectives mm.	Initial Magnification	OCULARS							
		4x	5x	6x	8x	10x	12x	15x	20x
48	2.2	8	11	13	18	22	27	33	44
40	2.8	11	14	17	22	28	33	42	56
32	4	16	20	24	32	40	48	60	80
30–22	2–4.5	4–9	5–12	8–19	10–24	15–35	18–43	20–48	30–70
25.4	6	24	30	36	48	60	72	90	120
16	10	40	50	60	80	100	120	150	200
12	15	60	75	90	120	150	180	225	300
8	20	80	100	120	160	200	240	300	400
5	36	144	180	216	288	360	432	540	720
4	44	176	220	264	352	440	528	660	880
3	60	240	300	360	480	600	720	900	1200
1.8	95	380	475	570	760	950	1140	1425	1900
1.5	109	436	545	654	872	1090	1308	1635	2180

FIG. 3.—Part of a table provided for use with microscopes. The first vertical column gives in millimeters the distance at which the objective focuses on the subject. Note that an oil-immersion objective working at a distance of 1.8 mm. would give a magnification of 380 when used with an ocular itself magnifying four times. With this oil-immersion lens what ocular would give a magnification of 570? Of 760? (Spencer Lens Company.)

much simpler to speak of the ocular, or objective, as if it were a single lens, and each has been treated as such in Figure 2.

Oculars and objectives are numbered by the manufacturers to indicate differences in magnification. Oculars, for example, may be marked x4, x8, x10, indicating that they magnify the object four, eight, or ten times respectively.

A common way of marking objectives is in terms of their focal length (2 mm., 1/12 inch), indicating either the distance of the lens from the object being examined, or the distance at which a simple lens would be held to produce the same-size

image. Oculars, likewise, may be designated in the same way (1 inch ocular).

In any system of marking, lower numbers on oculars indicate lower magnifications, and *vice versa.* In objectives, the lower the number, the higher the magnification: a 1/12 inch objective magnifies an object about 100 times, and a ⅔ inch objective magnifies only about 15 times.*

Some manufacturers, however, are beginning to number even the objectives in direct terms of magnification, so that anyone can readily compute the magnification being used; for example, an eyepiece marked x4 and an objective marked x95 would give a magnification of 4 x 95 or 380. In most cases, however, the student will find himself dependent upon the magnification tables provided by the various manufacturers (Fig. 3) for their respective microscopes. Roughly, the magnifications obtained with the ordinary compound microscope range as follows:

Low-power objective and low-power ocular	50–60
Low-power objective and high-power ocular	90–100
High-power objective and low-power ocular	250–370
High-power objective and high-power ocular	400–500
Oil-immersion objective and low-power ocular	510–700
Oil-immersion objective and high-power ocular	900–1200

2. MICROSCOPE PRACTICE

Position.—Place the stand so that the two arms of the base are directed away from you. Do not twist the microscope to the left or right, but keep it at right angles to the body, so that the screws, etc., will always have the same relative position, and may, therefore, be controlled without removing the eye from its position above the ocular.

See that the low-power objective is directly over the hole in the stage. As it moves into position, you can hear or feel it click into place.

Adjust the mirror so that light is reflected up through the tube and so, finally, through the ocular. Never use direct sunlight. The concave side of the mirror focuses as well as reflects the light which it receives, and it gives, therefore, brighter

*This holds wherever the focal distance is used to number the objectives (12 mm., 1.8 mm., ⅔ in.). In some of the older makes objectives bear arbitrary numbers (7, high; 3, low).

illumination, (except with the oil-immersion lens, which will be described later).

Mounting Objects.—Place a few fibers of cotton in the center of a *clean* glass slide. In the compound microscope we see by transmitted light; therefore, do not put so much material on the slide that the necessary amount of light cannot get through the mass. With a pipette put a drop of water on the cotton fibers. Cover with a *clean cover-glass.*

Focusing.—Using the coarse adjustment, raise the tube until the lower end of the low-power objective is about one-half inch above the stage. Place the mounted slide so that the object or material on it is in the *optical axis,* which is, for practical purposes, the axis of the tube. With the coarse adjustment, lower the objective *before* looking through the tube to within one-fourth inch of the cover-glass. Then while looking through the tube, slowly *raise* the tube until the object comes into view. Such adjustment of the optical parts is called *focusing.* Repeat the above operations until you are able to find the focus quickly with the low-power. Bubbles of air are often caught below the cover-glass. They may appear as iridescent globules or black rings or irregular, black-outlined areas. (Fig. 40.)

Never focus downward while looking through the microscope. When it is necessary to lower the tube, look directly at the end of the objective from the outside, keeping the eye level with the stage.

High-Power Objectives.—When an objective is in focus with the low-power, it is possible, by turning the nose-piece, to bring the high-power objective into the optical axis, and so nearly in focus that but a slight turn of the fine adjustment screw is needed to bring it into focus. The high-power lenses of different manufacturers, however, are not always interchangeable; the length of the different high-power objectives may vary so much that the objective may strike the slide or the wet cover-glass as it swings around into place. It is always safer, therefore, to raise the tube slightly before swinging the high-power objective into position. Great care must be taken in order to avoid touching the cover-glass with this objective. Why? (See pp. 8–9.)

Having secured good illumination with the low-power, raise the tube, as suggested above, and swing the high-power lens into place. Looking at it from the outside, the eye level with the stage, focus down with the coarse adjustment until the objective almost touches the cover-glass. Then, with the eye at the ocular, focus up *slowly* until the object comes into view more or less distinctly. Correct the focus with the fine adjustment screw, making such adjustment for light (mirror or diaphragm) as may be needed.

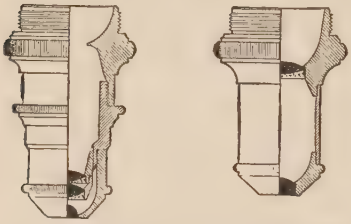

FIG. 4.—Sections through two objectives showing that each is composed of two or more convex lenses. These lenses may be convex on one or both surfaces. Note that the inner lenses are made of two different pieces of glass.

Practice changing from low to high power repeatedly, until the high-power can be adjusted quickly without contact with the cover-glass; this avoids wetting the lens or crushing delicate objects under the cover-glass.

When using the high-power objective, always turn or *lift* the nose-piece before removing the slide from the stage. Why?

Avoiding Eye-strain.—Keep both eyes open, and use the right and left eye alternately. If it is very difficult to keep both eyes open, cut a circular hole in one end of a one-inch strip of paper; slip the hole over the ocular, allowing the other end of the strip to extend across under the unoccupied eye. Gradually reduce the size of this strip until you can dispense with such aid entirely.

Care of the Microscope.—Clean the lenses with the thin Japanese lens paper provided; never use filter paper or a handkerchief. Use the lens paper for no other purpose and keep it protected from dust; discard promptly all used pieces. Clean the exposed lens surfaces before you begin work each day, and wipe them off carefully before you put the microscope away. If the high-power objective needs cleaning other than this, consult the instructor. (See p. 11 for alcohol and other solvents.)

Keep the mirror and ocular clean. With a soft cloth wipe dust from the metal parts of the instrument.

When lifting or carrying the microscope, grasp the pillar

below the stage. Hold the microscope in a *perpendicular* position, or the oculars may drop out.

Keep these directions at hand, and review them until you are familiar with the microscope and its workings.

3. ADDITIONAL MICROSCOPE EXERCISES

Reasons for Mounting in Water and Using a Cover-glass.—Take four slides and place a few grains of starch on each. (Potato starch grains are excellent for this purpose, as they are large and have very definite surface markings. Corn starch may be used, however.)

Cover the starch on one slide with a clean dry cover-glass. Add a drop of water to the starch on the second slide. Do the same with the third slide, but cover it with a clean cover-glass as well. Leave the fourth slide without water or cover-glass. Study these four slides in turn under the microscope until you can answer the following questions.

Which arrangement gives you the best results as measured by distinctness or sharpness of outline? By definiteness of surface markings? By flatness of field (more of the grains in focus at a given level)? What difficulty is found in the uncovered water mount? How should objects for study be mounted?

Permanent Mounts.—After examining the water mount of yeast stained with mythelene blue (p. 63), remove the cover-glass and dry the slide (room temperature). Then add a single

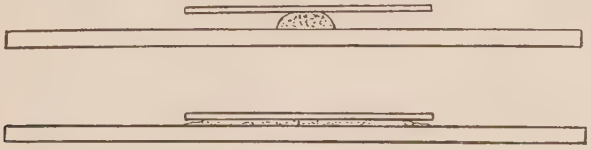

Fig. 5.—In the upper illustration, the cover-glass has just been placed upon the drop of balsam; its weight will slowly carry it down as shown in the lower drawing. Slight pressure (pencil eraser) will make sure that but a thin layer of balsam is left between the cover-glass and the objects on the slide, thus allowing the microscope objective to approach near enough to the objects to be studied.

drop of Canada balsam, and place on it (Fig. 5) a clean cover-glass. (See also p. 11.) The weight of the cover-glass will cause it to approach nearer and nearer the slide, until there is but a thin layer of balsam between the cover-glass and the slide. The

layer may be made even thinner by gentle pressure. Too thick a layer will make the distance between the object and the surface of the cover-glass too great for the high-power objectives which have very short focal distances.

4. THE OIL-IMMERSION LENS

(This exercise may be deferred until higher magnifications are needed: for bacteria, mold nuclei, etc.)

Note the small surface through which light enters in the oil-immersion objective, thus making it necessary to focus the rays from the mirror, to insure that as many as possible do enter the lens. This is done by an extra lens, the *condenser,* placed under the stage. Swing the condenser in and out of position in the microscope set up for this purpose, and notice the difference in illumination. Does the condenser affect the magnification?

With a lens as convex as the oil-immersion lens the magnification is much increased, but the attendant refraction (Fig. 6), means a corresponding loss in light. Since fewer light rays are passed through the ocular to the eye, the image is less distinct than in lower magnifications. It is therefore necessary to see that less of the light rays are lost *before* they enter the lenses—in their passage from the glass slide to the oil-immersion objective. Ordinarily the rays pass through the slide, water and cover-glass on into the air, and then into the lens (Fig. 6). Every change into a medium of different density (air, water, glass) means a change in the direction of the light rays, and a corresponding loss, due to rays that never enter the objective, or fail to focus harmoniously. To lessen these losses, a drop of oil,* having the same refractive index as glass, is placed between the cover-class and the lens, making a continuous glass-oil medium from the slide itself to the objective (Fig. 6). (Some workers put a drop of oil on the top surface of the condenser, thus eliminating the air between the condenser and the slide. This cannot be done with a microscope having an iris diaphragm above the condenser, without "gumming" or destroying the diaphragm.)

*For beginners, we often substitute liquid vaseline or mineral oil. While it is not quite so near the refractive index of glass as cedar oil, it works very well, and has the great advantage of not drying out and "gumming" the lenses. Demonstrations may be left under the oil immersion lens for several days with this oil.

The water between the slide and the cover-glass may similarly be replaced by oil or, in permanent mounts, by balsam.

In rapid routine work where smears are often made on the *slide,* instead of on a cover-glass, the cover-glass may be omitted entirely; the drop of oil is then placed directly on the stained smear and the lens lowered into it. Lower the objective, as

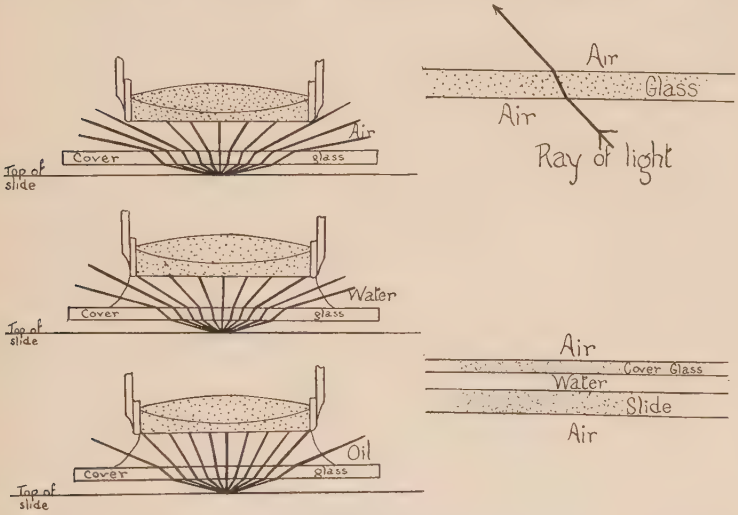

FIG. 6.—The upper right drawing shows the refraction of a light ray as it enters and leaves glass.

Dry and wet lenses. Changes in direction occur in light rays as they pass through substances differing in density. The three drawings on the left show the differences to be expected with air, water and oil respectively between the cover-glass and the objective. In which of these situations would more light rays finally reach the eye? Why is oil instead of water used in the wet lenses? (After Gage: The Microscope, Comstock Co.)

Show the changes to be expected in a ray of light passing through the layers in the lower right figure.

shown by the instructor, until the proper focal distance is obtained. When at work keep a thumb and finger on the fine adjustment, as already described (p. 3).

Do not use more oil than is necessary to make the connection between the slide and the lens. The lenses in the objective are set in balsam; solvents (chloroform, xylol, alcohol) which may be necessary to remove excess dried oil from a lens, may also loosen the lens from its setting or tarnish the polished metal surfaces. Oil-immersion objectives may be cleaned perfectly

without such solvents, using merely lens paper with *pressure*; two or more applications are usually necessary. Used pieces should always be discarded.

Good permanent slides cannot be made from oiled smears. It is very difficult to remove the oil without disturbing the organisms, and stained slides may lose their color if cedar oil is left on them. Permanent slides should, therefore, be mounted directly in balsam. If preferred, they may be mounted in water for preliminary inspection, and later dried and the balsam added.

REFERENCES

(See also general reference list in Appendix)

Carpenter: *The Microscope and Its Revelations*, Blakiston, Phila., 1901.
Gage: *The Microscope*, Comstock, Ithaca, New York, 1920.

REVIEW AND STUDY QUESTIONS

1. Define ocular, objective, low power, condenser, lens, diaphragm, reflector, index, magnification, oil-immersion and focal distance.
2. Why are water and cover-glass both necessary for good results with microscopic mounts?
3. How would you increase the size or area of the microscopic field?
4. How would you increase the apparent size of a single object (starch grain) under observation?
5. In your microscope how many ways are there of affecting the degree of illumination?
6. Does lengthening the draw-tube (to 160 millimeters) affect the apparent size of the object, the field or both?
7. Indicate the magnification used for one of the directions in Exercise 3 (Use the table on page 5 or 6 unless your microscope has a special table for that purpose).
8. Why should one hand be kept in contact with the fine adjustment screw?
9. Why might it be classed as a "criminal offense" to focus *down* while keeping the eye in position at the ocular?
10. Draw a series of three circles to show the relative sizes of the exposed surface of the lens as seen from below in the oil-immersion lens and in two lower power objectives.

CHAPTER I

GREEN OR INDEPENDENT PLANTS

It is unusual to begin a test on microbiology with a chapter on green plants, but green plants are familiar and bacteria are not, often as we have heard the word bacteria.

Our knowledge of a subject often gains definiteness by a negative statement or a contrasting background. This is particularly true with regard to bacteria and other micro-organisms. That they are "simple, dependent, and relatively undifferentiated organisms" means nothing to us unless we have for contrast fairly definite images of organisms that are complex, independent, and highly differentiated. Then only can we understand the problems associated with bacteria and their multiplication: whether we are dealing with useful micro-organisms, as in cheese-making or soil-improvement, or whether we are concerned with harmful forms, affecting our staple crops, our farm animals, or our own bodies.

The material assembled in this and the following chapter is worth while only as it illustrates and emphasizes:

1. Dependence or independence with regard to *organic* food materials;
2. Complexity or simplicity in cell structure; and
3. Interdependence of cells, or "division of labor" with consequent cell specialization.

At the end of this chapter, and again at the end of the next chapter, the student should review the material presented, and determine for each fact, illustration or class exercise, whether or not it falls under one or more of the three main headings in the preceding paragraph.

LECTURE AND READING OUTLINE

A. Manufacture of organic food (sugar) by green plants
   (A) Peculiar to green or chlorophyll-bearing cells
      a. Palisade tissue (Fig. 7)
         (a) Upper or sun-exposed side of leaf, below epidermis

(b) One or more layers of closely-packed chlorophyll-bearing cells

b. Spongy tissue

(a) Between palisade tissue and lower epidermis

(b) Loosely-packed cells, with irregular connecting spaces throughout

1. Spaces allow temporary storage of oxygen (O), carbon dioxide (CO_2), water vapor

2. Position of cells suggests series of braces (Fig. 7)

(1) Preserving storage spaces between cells

(2) Favoring rapid cell exchanges (O, CO_2, H_2O)

(c) Less sugar formed

1. Less green, less chlorophyll present

2. Less sunlight reaches spongy tissue

(B) Sugar manufacture a combination or synthesis of CO_2 (from air) and H_2O (from soil)

a. Process: $6\ CO_2 + 6\ H_2O \longrightarrow C_6H_{12}O_6 + O_{12}$

b. Requirements for this synthesis of CO_2 and H_2O

(a) Sunlight (photosynthesis)

1. Plants adapted to different intensities (shade-loving, etc.)

2. Sun as source of energy for utilization of CO_2

(b) Chlorophyll

1. Held in special bodies, or chloroplasts

2. Not soluble in water; normally retained in cell

3. Iron necessary for formation of chlorophyll and the synthesis of sugar

c. Relation of sugar manufacture to content of atmosphere

(a) Removes CO_2 from atmosphere

1. One yard leaf surface can use CO_2 from about 2,500 liters of air in 1 hour, making 1 gm. of sugar

Estimate air purification due to manufacture of one pound of sugar; and of one ton

(b) Renews atmospheric oxygen

1. Otherwise constant oxygen loss: breathing of plants and animals, burning of fuel, etc.
2. Copious elimination of oxygen by green plants
   (1) Twelve atoms of oxygen for every molecule of sugar formed
   (2) Sufficient to offset enormous amount of oxygen consumed. Oxygen content of air (21 per cent.) unchanged in over a century elapsed since air was first analyzed, despite tremendous increase in population and rise of fuel-consuming industries

d. Sugar changes in plant cells, non-green as well as green

(a) Change of simple sugars to double sugar, as cane sugar, $C_{12}H_{22}O_{11}$

(b) Change of sugar to starch—often implied in discussion of photosynthesis; but light not necessary for this change (*e.g.,* starch is formed in underground stems and roots)

1. Accomplished by common hydrolytic process: removal of H_2O; proportion $C_6H_{12}O_6$ becomes $C_6H_{10}O_5$
2. Regulates per cent. of sugar in solution in cell
3. Advantages in change of sugar to starch: starch does not interfere with osmotic pressure of cell, stores more compactly, ferments less readily

(c) Change of starch back to sugar

1. Reverse of (b) above; also a common hydrolytic process
2. Necessary before starch can leave the cell
3. Disappearance of starch from leaf cells over night thus explained
4. Change of this "transfer sugar" to storage starch in seeds, roots, etc., as in (b) above

e. Sugar in relation to protein production
   (a) Source of carbon for protein building
   (b) Used with minerals
      1. Absorbed with water by roots
      2. Available mainly as salts (potassium nitrate)
      3. Important minerals: nitrogen, calcium, potassium, iron, phosphorus, sulphur, magnesium, sodium
   (c) Plants ultimate source of all our proteins
      1. Directly, beans and other vegetables
      2. Indirectly (meat, milk, eggs) from plant-using animals

B. Green plants *independent,* in contrast with non-green plants (molds, bacteria), man and lower animals, all of which are dependent upon green plants directly or indirectly through plant-feeding animals. Plant independence shown in the food, oxygen and water relations
   (A) Originate energy-producing foods
      a. Sugars, by photosynthesis
      b. Starches, from sugars
      c. Fats and oils; man dependent here also
         (a) Directly: plant oils, such as palm, olive, corn, and cotton-seed
         (b) Indirectly, through plant-feeding animals, for lard and butter
   (B) Source of all tissue-building proteins (e above)
   (C) Minerals as food
      a. Plants can utilize inorganic form (salts, oxides, etc.) as obtained from soil
      b. Man dependent upon minerals as worked over by plants
         (a) Exception: table salt, only mineral food available to man without plant intervention
         (b) Values of certain foods related to mineral content (calcium in milk; iron in spinach)
   (D) Oxygen needed for respiration (oxidation processes in cell)

a. Process common to all living cells: plant and animal
b. Oxygen as source of energy
   (a) Essential cell activities
      1. Special work of cells (photosynthesis, absorption, etc.)
      2. Digestion of food itself
      3. Growth and repair of cells; assimilation of new substances
      4. Production of new cells
         (1) In growth of plant or animal
         (2) In reproduction
   (b) Obtained by
      1. Green plants
         (1) Through oxygen accumulating in green tissues and surrounding air as sugar is made from carbon dioxide and water
         (2) Oxygen used relatively small; excess diffuses into surrounding air
      2. Man and lower animals
         (1) From oxygen discharged into air by green plants
         (2) Animal needs heavy compared with plants; wholly dependent upon plant elimination of oxygen
   (c) Night and day relations (Fig. 19)
      1. Oxygen used constantly by all living cells, night and day
      2. Oxygen liberated by green plants during the day only
         (1) Surplus thus liberated sufficient for plants at night—for animals day and night
         (2) Plants not harmful at night (bedrooms), as oxygen demand and CO_2 elimination of plants are both small

c. Summary of CO_2 and O in relation to green plants

CARBON DIOXIDE

(a) Raw material used in photosynthesis
(b) Liberated by green plants constantly, day and night
(c) Amount liberated small
(d) Accumulation useful to green plants only

OXYGEN

(a) Necessary for metabolic processes of all living cells
(b) Liberated by green plants during the process of photosynthesis, during daylight only
(c) Amount liberated large, completely masking CO_2 elimination in day time, leading to popular misconception: "Plants breathe in CO_2"
(d) Excess O available for animal and plant respiration

(E) Water relations

a. Water, as solvent, carrying soil minerals into plants
b. Water taken by plants exceeds actual need as water
   (a) Necessary soil minerals are absorbed in very weak dilutions
   (b) Excess water eliminated (leaf)
      1. Eliminated as water vapor
      2. Rate surprising, may exceed plant weight on hot summer day
      3. Evaporation takes heat; cooling effect beneficial to man on hot summer days when evaporation rapid
   (c) Water rotation in plants
      1. Water eliminated by leaves condensed into dew and rain
      2. Absorbed by roots from soil with new load of dissolved minerals
      3. Illustrated small scale: fern globes, Wardian cases

(F) Summary of the green plant as an independent or self-sufficient organism

a. Can complete full life cycle using only inorganic materials as source of food and energy; animals and non-green plants must have as such sources food substances in organic form. All sugar, starch, protein, and fatty foods are traceable ultimately to plant origin

b. Supplies in photosynthesis the oxygen for its own metabolic processes, while animals and non-green plants depend upon green plants for their oxygen. Green plants use in photosynthesis a waste (CO_2) formed in respiration; in respiration in turn, green plants utilize part of the waste (O) formed by them in photosynthesis (Fig. 21)

c. Has also a water rotation, assuring a renewed supply of water with minerals

DEMONSTRATION AND LABORATORY WORK

While the following material has been selected mainly to illustrate the green plant as an *independent* organism, most of the exercises will also serve to illustrate the other two contrasts emphasized at the beginning of this chapter; and the directions given on p. 13 should be kept in mind during the following exercises.

1. Cross-section of a leaf magnified several hundred times (Fig. 7). Identify the main leaf tissues: upper and lower epidermis, palisade tissue, the looser spongy tissue, and one or more veins; locate them and at least one stomate in the epidermis, in the accompanying microscope.*

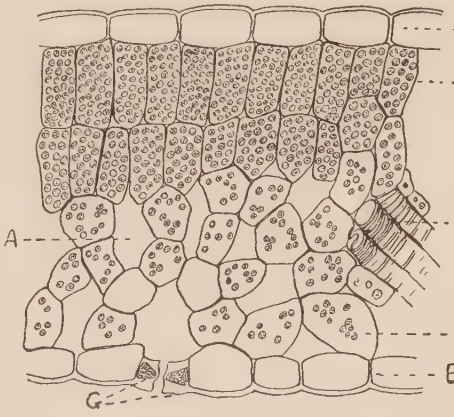

FIG. 7.—Cross-section of a leaf showing the colorless epidermis (E), the green palisade (P) and spongy tissue (S), a vein (V) and one stomate with its two boundary guard cells (G). (Broadhurst: Home and Community Hygiene, Lippincott.)

2. Crush a bit of green leaf (rotate eraser tip against cover-glass over water mount) and find under microscope one or more cells containing chlorophyll grains.

3. Strip off a bit of epidermis from the lower surface of a leaf and mount in water. Notice the transparent character of epidermal cells (light transmission to green cells).

* Throughout these demonstrations the specimens themselves are shown in the course as it is given in our laboratories at Teachers College. In most cases, however, the illustrations will give sufficient information to make the text understandable; and teachers using this outline may find it more broadening to use similar rather than identical illustrative material.

Move the slide around until you locate a stomate with its two curved guard cells (Fig. 8), which regulate the size of the

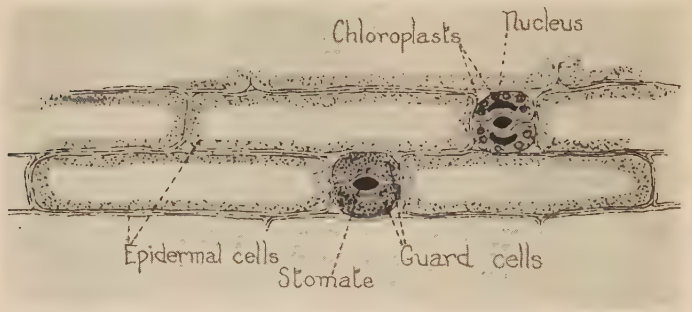

FIG. 8.—A piece of leaf epidermis stripped off and placed flat on a slide. Note the elliptical opening between each pair of curved guard cells. The elimination of water, and the constant exchange of CO_2 and O are possible because of these numerous openings in the leaf surface.

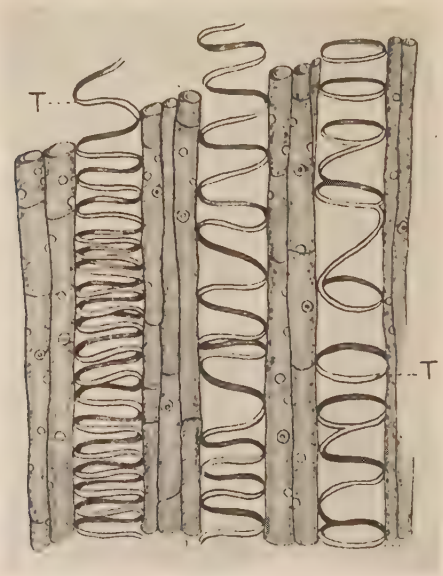

FIG. 9.—A leaf stem cut lengthwise shows such water tubes as these; special thickenings (rings, spirals as at T) prevent collapse of the water-carrying tubes. It is these tubes which rapidly become colored when stems are put into colored liquids.

central opening and the elimination of water. Of what advantage is the usual position of the stomates on the under side of leaves?

4. Place a (bleached) leaf of table celery in a glass of colored liquid (eosin, red ink). When the leaf begins to show the color, cut the stem across to determine the route of the liquid; cut down lengthwise through the red areas, or break the stalk to separate out the red stringy water tubes (veins).

5. A lengthwise cut through a leaf stalk, showing water tubes with ring-like or spiral thickenings in their walls (Fig. 9). How do such thickenings help insure the transfer of water to the leaf?

6. An experiment set up to show that leaves eliminate water (Fig. 10). The control shows that the water collected in the glass inverted over the leaves did not come from the enclosed air nor through the separating cardboard (See 3 preceding). Does the rate of water elimination occurring in this experiment make the statement that stomate openings "constitute one to three per cent. of the leaf surface" seem plausible?

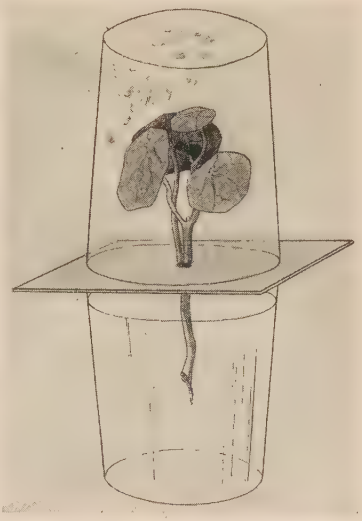

FIG. 10.—Leaf elimination of water: first from the cells into the spaces in the leaf and, finally, out through the stomates (see Fig. 7) to the outside air.

7. Starch formation by leaves.

(a) Starch and water and iodine give a characteristic color reaction when combined. The color varies from light blue to black, depending upon the proportions used (as you may demonstrate with the above-named substances, in the tray provided).

(b) If new to you, test for starch some of the plant substances in the accompanying dish: cracker, white potato, sweet potato, and bean seed.

(c) How may we tell for ourselves whether or not leaves contain starch? To see the characteristic blue or black color reaction, it will be necessary to remove

the chlorophyll or green coloring matter first. This is soluble in alcohol, and may be extracted by soaking the leaves in alcohol 6 to 24 hours. In the late afternoon pick two or three sun-exposed leaves from the plant and decolorize in alcohol until the leaves become white or yellowish white. (The process

FIG. 11.—As the starch in the leaf cells is turned blue or black, by the iodine, the leaves themselves become practically black. (To show this, complete removal of the chlorophyll is necessary: boiling before adding alcohol, renewing the alcohol, etc.)

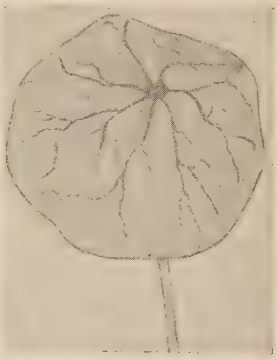

FIG. 12.—This leaf from which the starch has been transferred is but a pale yellow or yellowish-white, the color of the cells and the diluted iodine.

may be hastened by boiling the leaves in water for 3 to 5 minutes before decolorizing them.) Cover the bleached leaf with weak iodine; as it reaches the starch in the leaf cells, it turns the leaf black (Fig. 11).

8. Starch transferred from leaves during the night. This leaf (Fig. 12) was picked from the same plant as the one shown in Figure 11; but in the early morning of the following day (before any sunlight reached it) it was bleached and treated with iodine. Why does it not give the starch reaction?

This leaf presumably made starch (sugar) the previous day, just as its companion leaf did. By the following morning the starch had disappeared. A small part of the sugar or starch was doubtless used by the leaf cells themselves for energy, growth, etc., but most of the starch was transferred from the leaf to other plant parts during the night.

To leave the plant cells (to get through the cell membranes) starch has to change back to sugar. On reaching the other plant parts this transfer sugar may be again changed to starch for economical storage.

9. Sunlight necessary for photosynthesis. Part of this leaf (Fig. 13) was covered by a bit of tinfoil or cork; the rest of the leaf was then left exposed to the sun as usual. Explain the position of starch in this leaf.

10. Chlorophyll necessary for photosynthesis. A white-edged geranium leaf was picked at 4 P.M., decolorized, and treated with iodine. Which region (white or green) contains starch? What is a second essential for starch formation (Fig. 14)?

11. The under side of this leaf (Fig. 15) was covered with vaseline to prevent access of CO_2 through the stomates. What is a third essential for starch formation? What is one function of the stomates?

12. A red coleus (*Iresine*) leaf (Fig. 16) tested for starch. This shows that chlorophyll may be present and active, though masked by a second coloring matter. Such protective pigments are often present in tropical plants.

FIG. 13.—This leaf was decolorized and treated with iodine as in 7 (c) above. Why does the part covered by the cork show no starch?

13. A mass of water plants temporarily placed under a glass vessel (Fig. 17) to collect the oxygen given out as the plants use CO_2 to make sugar. The water medium shows the bubbles of oxygen, which escape unnoticed from the leaves of ordinary land plants.

14. Respiration in green plants.

Preliminary statement. To demonstrate respiration, at least one of the two phases of the process must be proven: either the exhaustion of O from the surrounding medium, or the addition of CO_2 to it. The latter is much easier to do, especially with green plants (since they liberate oxygen in photosynthesis).

The well-known lime water test for carbon dioxide may be used in demonstrating that a plant eliminates carbon dioxide. If not familiar to you, demonstrate for yourself by taking a glass of lime water, $Ca(OH)_2$ (or barium water, $Ba(OH)_2$), and adding to it CO_2. This may be done by running in a little charged water containing CO_2, by blowing your breath into the glass, or by simply leaving the glass of

FIG. 14.—The dark or iodine-reacting part corresponds to the green part of the leaf. What is the function of chlorophyll? (Thick leaves like geranium do not show the black iodine reaction as strongly as thin leaves like nasturtium.)

FIG. 15.—A small amount of carbon dioxide may pass through the cell walls themselves. Some carbon dioxide was present in the leaf spaces when vaseline was applied. Does the result show these sources sufficient for normal leaf action?

FIG. 16.—If the coloring is drawn out by alcohol, even with leaves which do not show any green color (ornamental bedding plants), it may be demonstrated that chlorophyll was also present.

FIG. 17.—The elimination of oxygen by green plants as they use CO_2 to make sugar (and finally starch) is most easily observed in water plants, where bubbles of oxygen may be seen rising from the plant. As shown here with Elodea, water in a test tube may be displaced by the oxygen as it is formed, thus enabling us to collect and test it (smoking match bursts into flame, etc.).

lime water exposed to the air of the room with its contained CO_2. The CO_2 unites with the lime water, making a whitish substance, calcium carbonate, Ca CO_2 (or barium carbonate, Ba Co_2), which may remain for

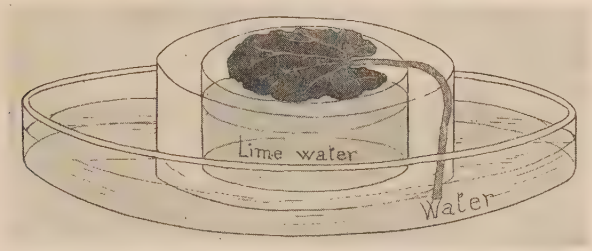

FIG. 18.—By exposing lime water near them, we may demonstrate that green leaves and other plant parts respire or breathe just as animals do. If the lime water under the leaf becomes milkier or has a heavier precipitate than the control lime water, what does that indicate?

a time on the surface as a definite film, cloud the liquid, or settle to the bottom as a friable precipitate.

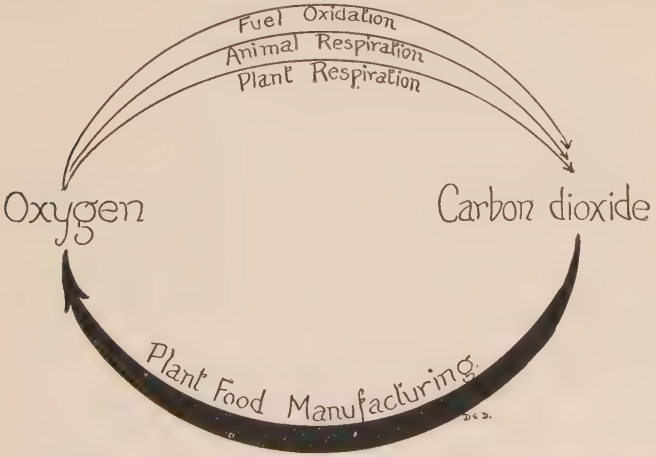

FIG. 19.—Green plants use so much carbon dioxide and liberate so much oxygen that the three big demands for oxygen indicated by the other half of the cycle are all satisfied. What becomes of the CO_2 produced by these?

How can we use this test to demonstrate that respiration takes place in green plant tissue?

In the above illustration (Fig. 18) a leaf has been placed near a small dish of lime water, the stem extending into the water of the larger jar. How can we make sure that the CO_2 which the

leaf gives out in respiration does not escape too rapidly to allow it to come in contact with the lime water?

Does the air originally enclosed in the inverted jar contain any CO_2? Of what value in this experiment is the water in the outside jar? Does it make any difference how large a dish we use to cover the lime water? How can we tell how much of the precipitate formed in the next 12-24 hours is due to CO_2 given off by the leaf? Why do we set up a control without a leaf?

REFERENCES

(See also general reference list in Appendix)

Bigelow: *Applied Biology*, Macmillan, New York, 1911. (Plant section.)

Curtis: *Nature and Development of Plants*, Holt, New York, 1921. (Chapter on The Leaf.)

Gager: *Fundamentals of Botany*, Blakiston, Phila., 1916. (Early chapters.)

REVIEW AND STUDY QUESTIONS

1. Define or describe chlorophyll, plant respiration, organic food, photosynthesis, oxidation, organic salts, protein and starch.

2. Show how the structure of ordinary leaves adapts the leaf to its work of sugar manufacture.

3. In how many ways are man and other animals dependent upon green plants?

4. What functions do the stomates play in the work that goes on in the leaf?

5. What processes do plant and animal cells have in common? Name at least one which is characteristically a plant phenomenon.

6. Trace the plant manufacture of sugar from the foundation materials: carbon dioxide and water.

7. What part do soil minerals play in food manufacture by green plants?

8. Find in the leaf illustrations examples of interdependence of cells or division of labor.

CHAPTER II

THE CELL

Cell studies, emphasizing modifications in structure, and giving material for illustrating (1) complex and simple organisms, (2) differentiation of tissue, and (3) division of labor, and relationship of structure to function.

Which of the green leaf exercises illustrates one or more of these points? Find additional illustrations from the following exercises:

LECTURE AND READING OUTLINE

A. Typical plant cells
   (A) Parts common to most living plant cells
      1. Wall
      2. Cytoplasm and its limiting membranes
      3. Nucleus and nuclear membrane
      4. One or more water reservoirs or vacuoles
   (B) Main contrasts with animal cells
      a. Cell wall and vacuole lacking in most types of animal cells (exception, vacuole, present in such one-celled animals as the ameba)
      b. Plastids common to many types of plant cells (see (E) following)

B. Morphology and function of plant cell parts
   (A) Wall
      a. Support; compare animal skeletons
      b. Modifications
         Increased thickness, effect upon absorption and excretion; upon life of cell; rôle in life of plant as a whole
      c. Varieties of substances deposited; lignin (wood), cellulose, chitin, wax, mucin

d. Modifications affording both absorption and support: thin areas between thickened regions (Fig. 27)

e. Cell turgidity, as in animal cells, a factor in retaining cell shape, resisting pressure of adjacent cells; wilting rate of young and old seedlings, leaves, etc., related to thickness of walls

(B) Cytoplasm or cell body

a. Viscous mass, more or less transparent in character, surrounding nucleus

b. Composes bulk of young cells

(a) Vacuole often forms relatively greater volume in older cells (Fig. 30)

(b) Normal position, lining layer inside cell wall

c. Movement often visible, occasionally as active streaming (Fig. 37) through cross strands and around edges of cell (circulation)

d. Limiting membrane (cytoplasmic membrane)

(a) Present where cytoplasm is in contact with other parts of cell (cell wall, vacuole, nucleus)

(b) Selective absorption power of cell depends on semi-permeable character of cytoplasmic membranes, as well as chemical affinity of cell for substances available for absorption. Permeability affected by minerals (potassium, calcium)

e. Physiological activities of cytoplasm: absorption, excretion, enzyme activities (covering energy production in cell); some synthetic work (manufacture of starch by denucleated cells of spirogyra). Life in sense of continued existence is possible without nucleus (red blood cells live 10 to 30 days after losing nucleus)

(C) Nucleus

a. Initiates and controls synthetic metabolism; indispensable for growth, regeneration and reproduction (cell division)

(a) Growth and regeneration: impossible without nucleus; illustrated by experiments with protozoa (Stentor) having elongated necklace-like nucleus; when cut into fragments, only the parts with a portion of nucleus regenerate, others perish

(b) Chemically active; oxidation more rapid in nucleus than in cytoplasm

b. Structure

(a) Shape of nucleus. Spherical or ellipsoidal commonly; affected by pressure (storage material, such as starch; size of vacuole) or other special conditions

(b) Size, green cells, 5–25 microns (micron = one twenty-five thousandth of an inch) in diameter; in most molds less than five microns

(c) Nuclear membrane, limiting layer between nucleus and cytoplasm; nucleus occasionally not so distinctly delimited from cytoplasm, and existing as scattered chromatin throughout cytoplasm, as in most bacteria, blue-green algæ and some protozoa

(d) Nucleolus. One or more in most cells, differing in staining (usually with acid dyes); may contain vacuole and varying layers or may be homogeneous. Functions probably explained by considering it as mass of accumulated material for metabolic processes of nucleus

(e) Nucleus. Foundation a liquid (nuclear lymph) containing more dense gel, network, or reticulum, often called achromatin. Chromatin more or less evenly distributed throughout this network; sometimes described as of two kinds

1. Poor in phosphorus, taking acid dyes, and
2. Mainly nuclein, rich in phosphorus, and taking basic dyes. This staining distinction apparently may vary with phosphorus addition or loss.

c. Cell division
   (a) Mitosis type characteristic of higher plants and animals now known to be quite common throughout the living world; note nuclear changes (Fig. 35) in higher plants
   (b) Other types of cell division: constriction (molds), budding (yeast), as types of direct nuclear division (Fig. 36). Such direct division less certain to yield like inheritances to resultant cells. Direct division may occur in plants having also more complex mitotic division; budding of yeasts (direct) and spore formation of same yeast (mitotic). At present, observation of mitosis in protozoa (paramecium) prevents crediting division by mitosis to higher organisms only

(D) Vacuole
   a. A name, meaning vacuum, given when this reservoir was thought merely empty space
   b. Contents water plus organic acids (malic, formic, acetic, oxalic) and salts (of such acids); probably also inorganic acids
   c. Size relatively larger as cell matures (Fig. 30)
   d. Function
      (a) Ready water reservoir necessary because of
         1. Frequent and rapid hydrolytic changes needing or producing water, as in green cells, or
         2. Oxidation processes producing water
      (b) Storage of various organic substances, (see b above)
         1. Sugars, tannins, proteins
         2. Pigments—red and blue; some produce color changes with alteration in reaction of vacuole liquid; litmus-like changes in hepatica (blue to white to pink, as cell changes from slightly alkaline to neutral to acid); responsible for color of blue, red-blue and purple flowers (violet, iris)

(E) Other parts found in plant cells

a. Plastids. Differentiated portions of cytoplasm; definite process so delimited that plastids may be considered distinct cell organs, much as nucleus itself. Plastids have power of growth, and division; found in practically all higher plants; lacking in animal tissues, bacteria, and many fungi

(a) Number. One to many to a cell

(b) Shape. Normally more or less disk-like. Form, however, variable, Fig. 26; (differing in original shape and changes produced by substances stored in them)

(c) Types of plastids

1. Chloroplasts of green plants, composed of oily material holding chlorophyll; considered "world's ultimate food producers" because necessarily beginning stage (photosynthesis) in the formation of all organic food material
2. Other colored plastids containing various pigments: yellow (carotin, brown seaweeds); blue (phycocyanin, blue-green algæ), and red (phycoerythrin, red algæ). More than one pigment sometimes present; *e.g.*, green chlorophyll also present in red and in brown seaweeds, tropical land plants, etc.
3. Plastids as storage organs for food

(1) Leucoplasts: sometimes ameboid in character; may represent young stages of starch-storing plastids or colored plastids; starch plastids (Fig. 32) varying in size and shape with method of depositing starch; difference in water content of layers may give ringed or concentric appearance and explain radial cracks, especially in dried grains

(2) Colors other than green probably fatty substances (lipoids), not always in definite plastids; often crystallizing in curious and varied shapes, or seen as solid granules. Present in leaves, fruits, and yellow and red flowers

C. Cell activities

(A) General

a. Common to all plant cells; a partial list includes:

(a) Absorption of water and substances in watery solutions (minerals, oxygen, foods)

(b) Excretion of water and wax and other productions, also in solution (water, carbon dioxide, foods for transfer, enzymes)

(c) Synthesis of food substances into cell substances, stored foods

(d) Formation of special substances, useful in cell metabolism or in maintaining normal conditions of living substance (enzymes, crystals, pigments)

(e) Growth and repair, with all attending assimilation changes

(f) Oxidation or splitting of food materials to obtain energy

(g) Reproduction or multiplication of cells. Cell division (see mitosis above)

(B) Relationship between structure and function

a. Close; structure limits function, and function affects structure

b. Illustrative modifications

(a) New or additional parts

1. Green plastids for formation of sugar from inorganic carbon dioxide and water
2. Storage plastids, starch grains, for reserve storage (seeds, roots)

(b) Modification of part common to all plant cells

1. Cell wall, enormously extended and very thin in root hairs; texture and extent favoring absorption (Fig. 29)
2. Vacuole making main bulk of cell in such root hairs favoring water storage

(c) Storage of inert substances (starch) interferes with life of cell, and transforms it to mere storage area (Fig. 32)

(d) Complete change in structure and function of cell, as in water tubes, once living cells, but changed to mere tubes (living contents lost) and serving for mechanical transfer of water

D. Common mistakes in concepts of cell

(A) Two instead of three planes: corrected only by proper handling of the microscope; depth essential to an idea of the cell as a living mass of material

(B) The cell as morphological rather than living physiological unit: latter character probably more important; however, each limits the other, neither independent

(C) Confusion of cell parts with cells as a whole; a large number of students mistakenly speak of the "wall" and "nucleus" of a starch grain; or of the "cell division" of a chloroplast

(D) Such over-emphasis of the various component cell substances (chromatin, lignin, etc.) that a plant cell fails to be comprehended as a *protein unit*, with a high percentage of water (in the cytoplasm and vacuole) surrounded by a (practically) carbohydrate envelope or cell wall, and containing more or less stored food substance (sugars, starches, fats, and proteins)

DEMONSTRATION AND LABORATORY EXERCISES

1. Study of a living cell with well-defined parts. Strip a bit of epidermis from one of the inner scales of a good firm

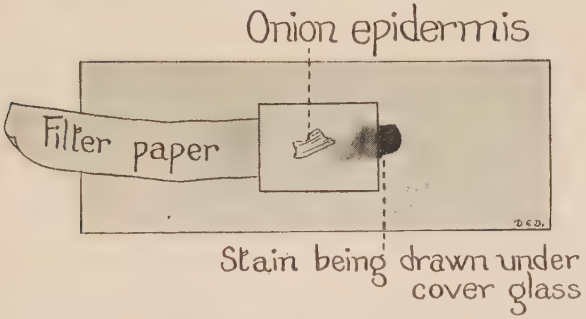

FIG. 20.—Instead of lifting the cover-glass and possibly disarranging delicate mounts, draw under new solutions (eosin, salt, fresh water), as shown here.

onion. Mount directly in water. Lower the cover-glass slowly and obliquely to avoid including air bubbles. Examine (low and high power). Why does the curvature of these leaves make a small piece better for mounting than a large piece? Draw iodine-eosin under the cover-glass with a strip of filter paper (Fig. 20).

Draw (on unlined paper) 3-4 adjoining cells, making each cell at least one inch long. Label each cell part: cell wall, cytoplasm (cell body) and nucleus (Fig. 21). Avoid dead or dying cells, which show a disintegration of the nucleus. Focus carefully on the nucleus; one or more definite bodies may be distinguished (nucleoli). These are important, in this exercise, not so much from the point of cell structure, as to insure that

you are using the microscope with sufficient skill. Practice with the fine adjustment screw until you gain some idea of the depth of the cells (third dimension).

2. Most plant cells have a distinct water reservoir. Examine again your slide of the onion epidermis, and find a region stained just enough to show a distinction between the denser cytoplasm lining the cell and the central watery vacuole. Sometimes there are cross strands of cytoplasm more or less dividing the vacuole.

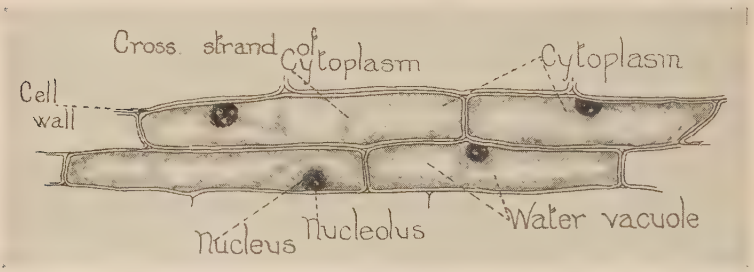

Fig. 21.—Normal epidermal cells of the onion. Note the paler central area in some of your stained cells; the vacuole shows less stain than the cytoplasm lying between the vacuole and the wall.

(Such strands allow more rapid transfer or distribution of material absorbed at one end or side of the cell.) Show the vacuole in at least one of the cells in your drawing.

3. Plasmolysis. To the same stained onion mount add ten per cent. salt solution. (Draw it under as you did the eosin.) Select only live cells with definite nuclei for observation and watch these cells for changes (Fig. 22), showing such changes by carefully labelled drawings.

What cell part hitherto unnoticed now becomes evident? Why?

Replace the salt solution with water. Do any of the plasmolyzed cells recover? All? Extreme or prolonged plasmolysis increases the concentration of salts, acids, etc., normally present, and may irritate cells beyond recovery.

4. In the above experiment (No. 3) water apparently passed out through the cell membranes (wall, cytoplasmic membranes) more rapidly than the salt passed in. Since water molecules are

smaller than salt molecules, what light does that throw on the plasmolysis of the onion cell? Proteins have very large molecules. Why did the cytoplasm (protein) stay inside the plasmolyzed cells while the water passed through?

5. The cell contents, especially salts, sugars, and acids, have an affinity for water. Show that the power of cells to draw in

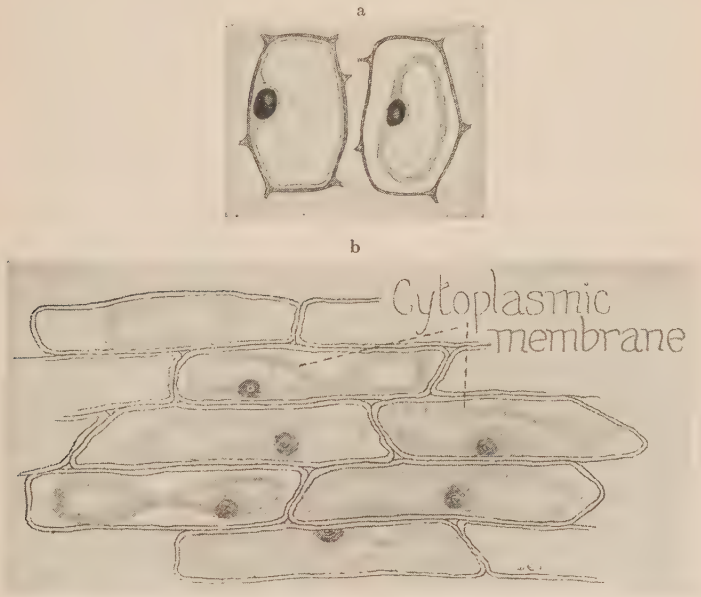

FIG. 22.—(a, b) By surrounding the onion cells with stronger salt solutions than they themselves contain, such changes as those shown in the lower cells may be obtained. Do the nuclei and vacuoles always show as distinctly as before? Compare also with the drawings above where the vacuoles show distinctly. Could such a change be produced by drying or wilting?

water is illustrated by the following experiment in which a glass tube (Fig. 23), partly filled with sugar solution and having a piece of membrane (the seed coat of a large bean, preferably the flat brown English bean, the broad Windsor bean) tied tightly over the lower end, is placed in water.

Note the original level of the sugar solution at the hour indicated on the label; compare with the present level of the liquid. What effect did the sugar inside the membrane have on

the water outside? In this experiment, we have a strong sugar solution on one side of the membrane, and nothing but water on the other. Since sugar molecules cannot pass through the bean membrane, how can the attraction of water and sugar be satisfied? Why might green leaf cells manufacturing sugar burst if they did not change their sugar to starch? Give one reason why it is

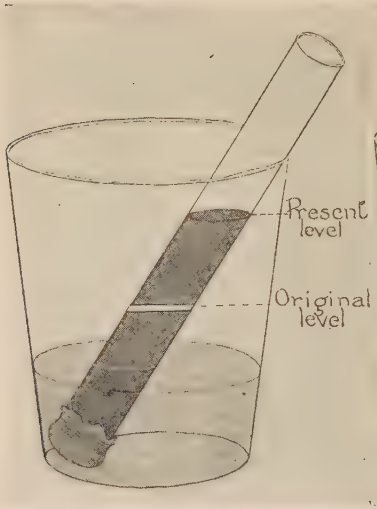

FIG. 23.—This tube with a membrane is sometimes called an artificial cell. It is really a very poor cell, as it (1) has inelastic walls (glass tubing); (2) is permeable only for a small part of its surface (bean membrane); and (3) has not a complete wall (open end to allow for expansion as liquid passes through the membrane).

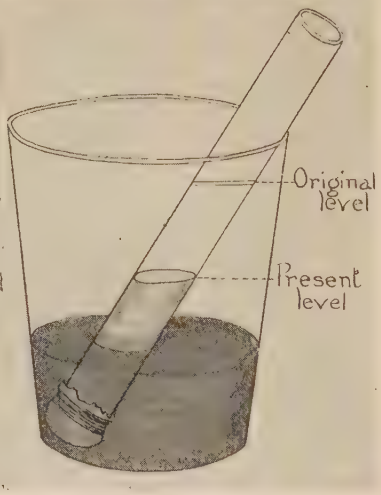

FIG. 24.—Water leaves this tube or artificial cell just as it passes through the onion membranes in the plasmolysis experiment. Which of the slices in Exercise 9 does this parallel?

better to use physiological salt solution instead of distilled water in making up blood loss; what effect might the injection of large amounts of water have on the blood corpuscles?

6. Reverse the experiment just discussed, as shown in Figure 24, to illustrate the passage of water from a cell, (No. 3 above). Why do roots die when in contact with strong salt solutions (brine from cream-freezer on grass)?

7. Which of the above exercises, 5 or 6, illustrates how root

cells (root hairs) absorb water from the soil? Which explains why bacteria are killed by salt solutions (salt as a throat gargle)? Which illustrates how molds and bacteria absorb water (from foods, animal tissues, etc.)?

8. The size of molecules is not the only factor that affects the rate at which substances diffuse through membranes. The relative proportions are also important. Given a membrane separating a strong and a weak solution of the same salt, we should have on one side a higher percentage of salt molecules, and on the other a higher percentage of water molecules, coming in contact with the membrane. In other words, on one side there would be a greater proportion of salt particles than water trying to get through; on the other there would be a greater proportion of water than of salt trying to get through. Leaving size out of consideration, from the side with a greater proportion of water molecules in contact with the membrane more water would pass through; while from the side with a higher proportion of salt in contact with the membrane, more salt would pass through.

Through such a membrane, salt and water would be passing in opposite directions at the same time. Could a green leaf cell be absorbing water and giving up sugar at the same time? Could a cell be absorbing calcium salts at the same time that it is giving out nitrate salts? Could a cell be absorbing oxygen and giving up CO_2 at the same time? Could a cell absorb oxygen, calcium salts, nitrate salts, and sugar at the same time that it is giving out CO_2, lactic acid and water?

9. Soak slices of potato or beet, of equal size and thickness, for three or four hours in plain water and in saturate salt solution. Feel the pieces, bending them slightly, to see the great difference in rigidity caused by these two liquids. Select from the illustrations in this chapter drawings that might be used to show the differences to be observed in the cells themselves if examined under the microscope.

10. When a cell loses its turgidity, becoming plasmolyzed, the cytoplasmic membrane no longer occupies its normal position close against the cell wall (Fig. 22). Such cells do not absorb or transfer substances normally, and the normal metabolic proc-

esses are interfered with. Besides, as mentioned earlier, the loss of water may mean too high a per cent. of acids, etc., in the cell. If plasmolysis is too extreme, the death of the cell follows. Did your plasmolyzed onion cells recover? Why? Does the time that elapses before wilted flowers are put into water affect their recovery? If bacteria and molds are dried too long, why do they fail to grow even when placed in favorable material?

11. The interior of this carrot (Fig. 25) was hollowed out and filled with sugar (or molasses). The top was then plugged with a cork having a glass tube in it. The carrot was immersed in clean water. What change is taking place in the sugar-filled cavity? What change is taking place in the cells next to the sugar-filled cavity? In the outer cells of the carrot? Why does not the outside water surrounding the carrot gain sugar? What advantage may cells derive from sugars or other substances which cannot pass through membranes as readily as water?

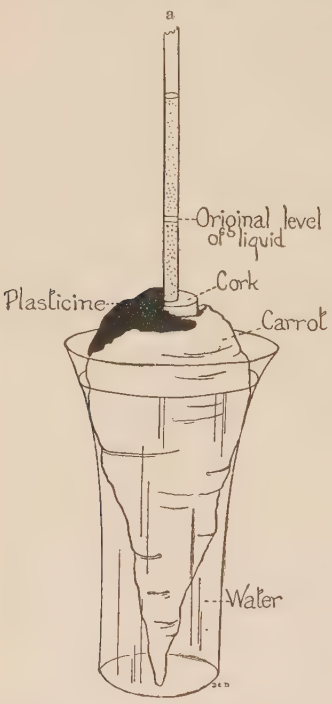

FIG. 25.—(a) Does this experiment (Exercise 11) resemble Figure 23 or Figure 24? (b) What is happening in cell 1? In cell 2? In the cell next the sugar-filled cavity?

12. In the above exercises the process and applications of osmosis and dialysis have been illustrated, without using either of these terms or the more technical terms, such as hypertonic solution, ionization, and osmotic pressure.

In the latest dictionaries you will find osmosis defined as including

(1) passage into and out of the cell; and (2) the passage of water only or of substances in solution, such as sugars and salts.

The direction (in or out) is not emphasized now by most people. Many text-books, however, distinguish between the passage of water and the passage of substances in solution (salts, sugars, etc.), calling the passage of water through a membrane, osmosis, and the passage of the sugars, salts, etc., dialysis. Others define osmosis as the passage of the solvent (usually water, of course); and dialysis, as the passage of the solute (sugars, salts, etc.).

Whatever terminology is used, the following introductory statements will be of value: a. Substances in solution tend to distribute

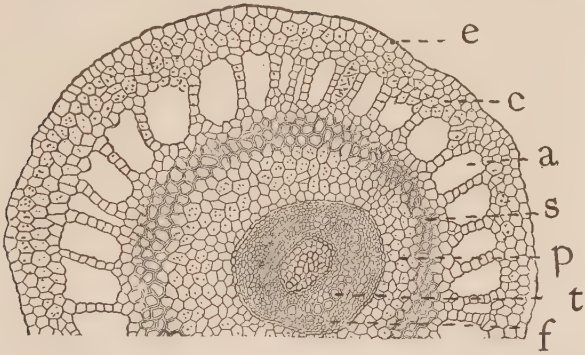

Fig. 26.—Morphological differences as described in the text (Exercise 13) show in this cross-section of the stem of a fern (Marsilia): e, epidermis; c, the underlying tissue, often containing chlorophyll; a, spaces (not characteristic of all stems); s, thick-walled supporting cells; p, ordinary parenchyma cells filling in between the more specialized cells; t, a ring of large tubes carrying water; and f, a surrounding layer of food-carrying tubes. (After Green: Vegetable Physiology, Churchill.)

themselves equally. b. This equal distribution of one or more substances throughout another is called diffusion. c. When this diffusion is hindered by a membrane (as in the various experiments above and in living cells) we call the process osmosis or dialysis (as discussed above). d. With a membrane in the way this exchange is modified

(1) Slower

(2) More or less "selective," depending upon the size of the molecules of the various substances when compared with the ultra-microscopic pores of the membrane

13. Cross-cut of a plant stem (Fig. 26).

This and most of the following slides are thin sections cut across or lengthwise through the part of the plant described. The various cells or tissues, as may be seen, vary in shape and size. Many of these slides have been stained with two or three different stains (mixed or else applied in sequence) and the fact that, in a given stained slide, some cells appear yellow, for example, while others in the same slide are

brown or red or green or blue, shows that they are different kinds of cells, differing in their chemical affinities as well as their physical shapes and proportions.

Move the slide freely and list at least four or five different kinds of cells in this section, as determined by the previous explanation. Look for differences in size, shape, thickness of walls, presence or absence of contents and unusual types of cell

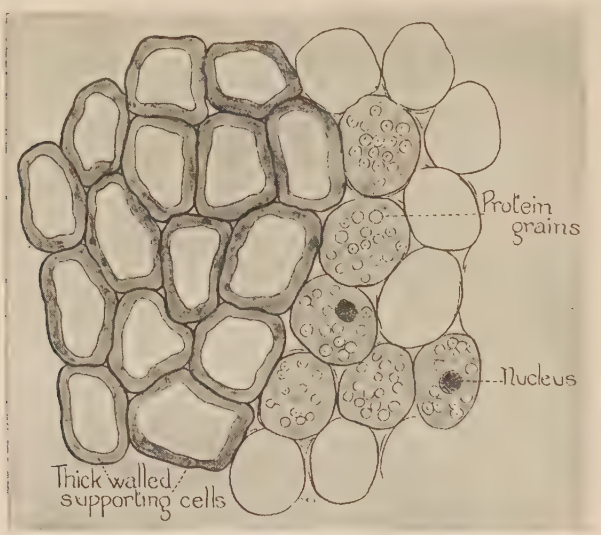

FIG. 27.—On the left some of the thick-walled supporting cells (s, in Fig. 26) found near the center of the stem (cross-section); on the right are the thin-walled cells (p, in Fig. 26), some of which show cytoplasm nuclei and smaller protein granules (see Fig. 32 right).

contents and coloring. Some cells are accidentally empty because in making such sections we cut *through* many of the individual cells as well as between them. Distinguish between the cause of such empty cells and the empty cells described in the next paragraph.

14. Near the center of this fern stem (Fig. 27) are cells so thick-walled that they are practically solid. How would this affect the transfer of materials into and out of such cells. The life of the cell? In the stems of the larger plants certain thick-walled cells lose their contents and become water conductors;

others serve only for support, giving rigidity to the stem. Which of the three headings on page 13 do such thick-walled cells illustrate?

15. This woody stem (pine) has been cut lengthwise (Fig. 28) through the water tubes or woody part so as to show the long units that make up the water-carrying tissue. Since water must be transferred, not only upward but outward through the tissues at every level, plants often have special adaptations for allowing the escape of sufficient water horizontally. Note that the side walls of these tubes have peculiar thin areas, which allow such escape of water and substances in solution without wholly interfering with the main upward flow of the water.

Fig. 28.—Thick-walled tubes of pine cut lengthwise and showing the thinner areas which allow rapid movement of water across the stem. Adaptation for support (thickening of the wall) does not, in this case, interfere wholly with water passage.

16. The root hairs shown here (Fig. 29) have a very thin cell wall. They differ from most of the live cells we have seen, in having an extremely large water reservoir, the cytoplasm forming but a thin lining inside the cell wall. Such large water reservoirs allow rapid absorption of water, and temporary storage of water before it is transferred to other parts of the plant.

17. Cross-cut of a piece of onion root (Fig. 30) taken near the tip, showing rather solid-looking young cells to the left, and older cells with large vacuoles on the extreme right edge of surface of the root. The older cells are pushed to the outside as new cells form. What change do you notice in the relative size of the vacuole in the young and the older cells? Compare with cells in Figure 29.

18. Most green plastids are coin-shaped. A common fresh water plant (spirogyra) has curious ribbon-shaped plastids twisted around inside the cell wall. The centers for starch

storage are indicated here by darker dots at intervals in each of these ribbon-like chloroplasts (Fig. 31).

19. Plastids may serve solely for starch storage. In the white potato these plastids become greatly enlarged and changed in shape by consecutive deposits of starchy material, giving the

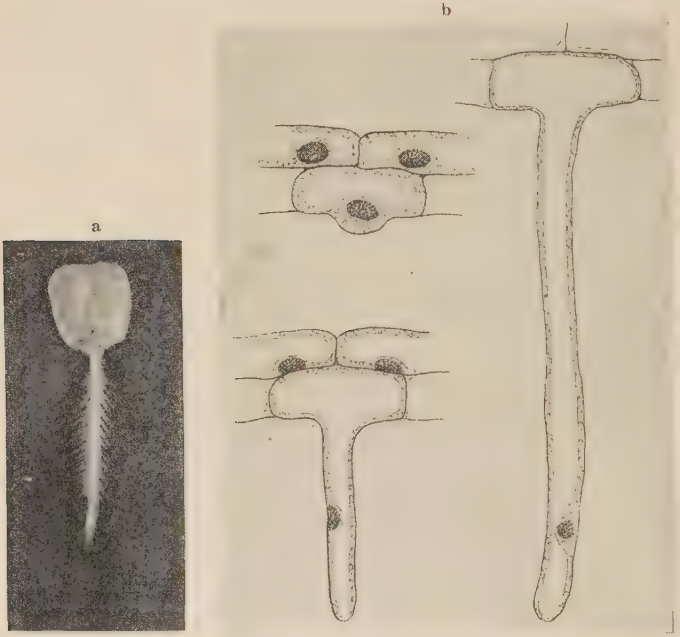

FIG. 29.—(a, b) Root-hairs. Corn seedling on the left (about natural size) showing root-hairs. On the right is shown (much enlarged) the development of a root-hair. Note that as the cell develops the vacuole becomes proportionately larger.

characteristic eccentric starch grain found in potatoes (Fig. 32). Mount potato, corn, and wheat starch (Fig. 32) in water and compare them: (a), shape; (b), size; (c), markings indicating layers of starch; and (d), other characteristics, such as cracks (age, drying).

20. Cells of a wheat kernel showing two kinds of storage grains, starch and protein. (Fig. 32.) Less definite food granules will be found later in yeasts (glycogen) and bacteria (metachromatic granules).

21. An illustration of enzyme activity. A little diastase was added to starch grains producing the morphological changes seen in Figure 33. What chemical change has occurred? How could it be demonstrated?

These serve but to illustrate the many and varied enzyme activities that take place in all living cells. Later, add here at least one illustration for yeasts; another for bacteria.

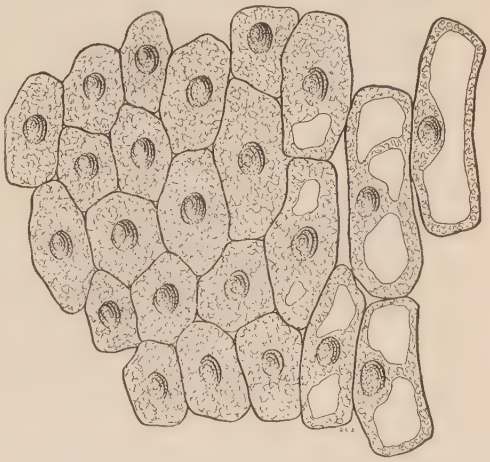

FIG. 30.—Another illustration (cells near root tip) of the characteristic increase in the size of the vacuole as plant cells grow older and larger.

22. All minerals absorbed by plant cells are not used directly in making cell substance. Excess minerals are sometimes combined with acids occurring in the cell, forming crystals, as shown in the trillium stem (Fig. 34), where bundles of needle-like crystals of calcium oxalate fill some of the cells. Later in the course, look up sulphur bacteria and determine the reason for their name.

23. Nuclear changes seen during cell division in ordinary plants. Compare the stages to be found in this root tip slide (onion, hyacinth) with Figure 35. Compare this process as described in the legend with the simpler one occurring in yeasts (Fig. 36).

24. Constriction of the nucleus (Fig. 36), a common occurrence in cell division of simple cells, such as amebæ and yeasts.

25. Movement within the cell. When a cell contains chlorophyll grains, they are sometimes carried along by the moving cytoplasm, making the movement of the cytoplasm more evident. Elodea, a common aquarium plant, has such thin leaves

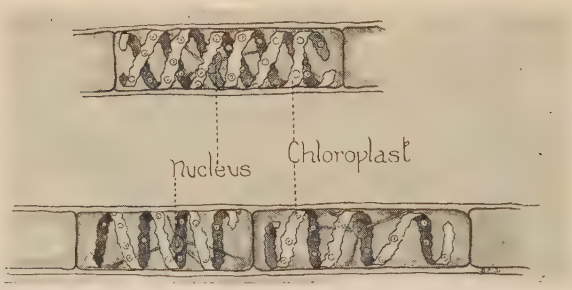

FIG. 31.—Spirogyra, a thread-like plant; the cylindrical cells contain one or more long flat chloroplasts, twisted ribbon-like inside the cell. (See also Fig. 69.)

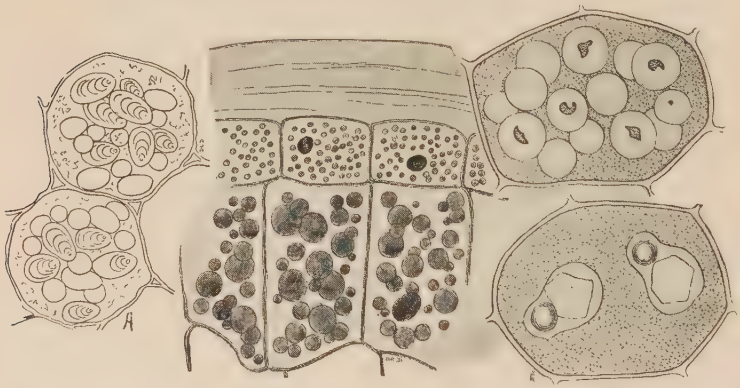

FIG. 32 a.—Plastids: (1) On the left are potato cells (A) in which the nuclei and cytoplasm both disintegrated as the cells became storage places for starch grains. (2) In the center is part of a section through a wheat grain, showing the thickish outer covering; next, three of the cells which store protein, and below, the inner starch-bearing cells. (See 32 b following.) (3) On the right are two cells (upper, fern; lower, castor oil) showing protein-storage bodies.

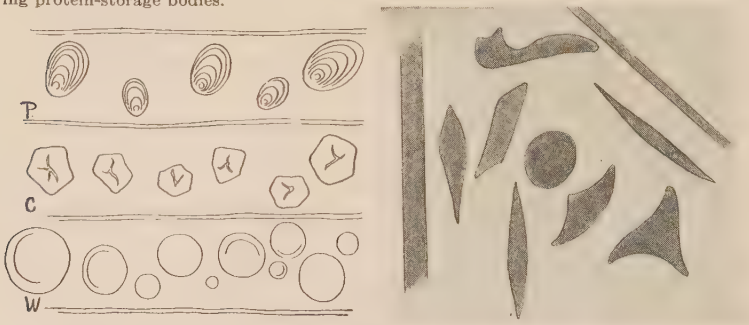

FIG. 32 b.—On the left starch plastids drawn to the same scale: P, potato; C, corn; and W, wheat. On the right are various chromoplasts connected with color in plants (carrot, yellow and red flowers) which apparently store fatty substances, which often crystallize or form very irregular bodies as shown here.

(but a single layer of cells, except near the midrib) that this cytoplasmic movement is very easily demonstrated (Fig. 37).

Mount a leaf of Elodea in water, and locate one or more cells

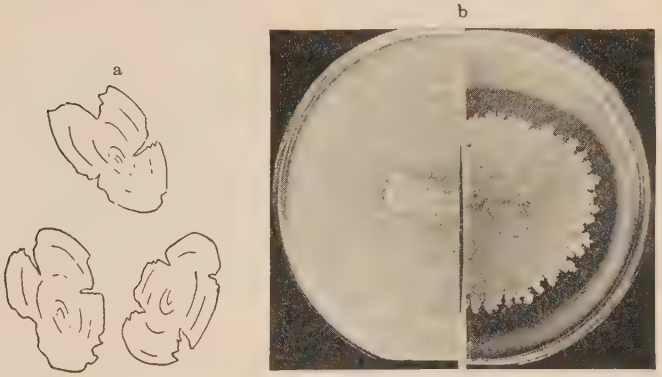

FIG. 33.—a, Physical evidence of enzyme action is afforded by the eroded or partly digested starch grains (Compare Fig. 32 b, which is drawn to a smaller scale). b, In the agar plate photographs (left *Sarcina*; right *Proteus*) only the *Proteus* organisms have digested or destroyed the milk mixed with the agar. (Green.)

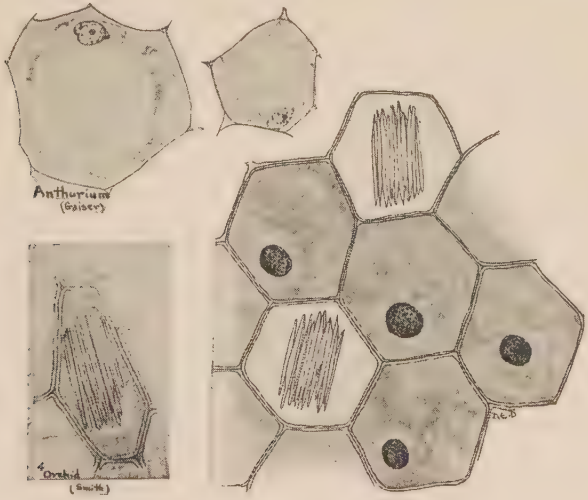

FIG. 34.—Various types of crystals found in plant cells. Find one crystal lying in the vacuole; one which is encroaching upon the whole cell area; locate one cell in which all living substances (nucleus, cytoplasm) have disappeared. (Bulletin Torrey Botanical Club: star-shaped crystals, Gaiser, in *Anthurium;* needle-like crystals, Smith, in orchids; drawing on right, *Trillium.*)

which show distinctly the movement of the cytoplasm. Focus into the middle of the cell and see how many seconds it takes for the cytoplasm to make a complete circuit. Is the rate affected

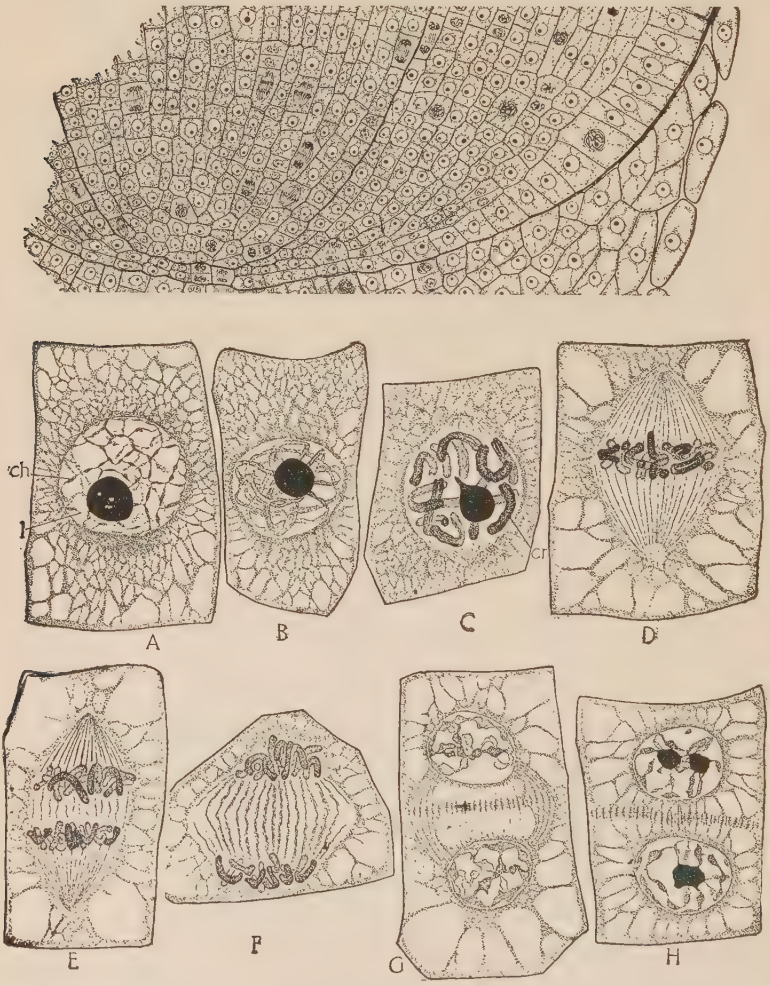

Fig. 35.—Eight different stages in nuclear changes characteristic of cell division in higher plants (mitosis). How many of these can you find in the part of the root tips shown above. Do lower plants, such as molds, ever show such nuclear changes in cell division? (See Fig. 44.) (Cardiff: Curtis's Nature and Development of Plants, Holt.)

by the magnification of the microscope? The time for a complete circuit?

26. Movement of cells as a whole. The accompanying illustration shows four photographs of an ameba (Fig. 99, II), taken

Fig. 36.—Simpler types of nuclear changes occur in cell division in one-celled organisms: yeast (left) and ameba (right).

from a moving picture film at such intervals that they represent time divisions of fifteen seconds. Note the change in shape:

Fig. 37.—A few cells from a leaf of Elodea (Fig. 17) showing chloroplasts in the cytoplasm lining of the cells. In active vigorous cells the cytoplasm may be observed in definite circulation inside the cell wall, carrying the chloroplasts with it; the circuit may be completed in thirty seconds or less.

the extension of the cell itself into an "arm or foot" on the upper side and the consequent withdrawal of the cytoplasmic substances from the lower left and right edges of the organism.

Such movement of the whole cell is more characteristic of animal life (protozoa) than of plant life; the firm wall of plant cells does not allow such changes in shape as shown in this ameba.

27. Paramecium with numerous cilia or appendages which make locomotion possible by a kind of whip-lash movement of

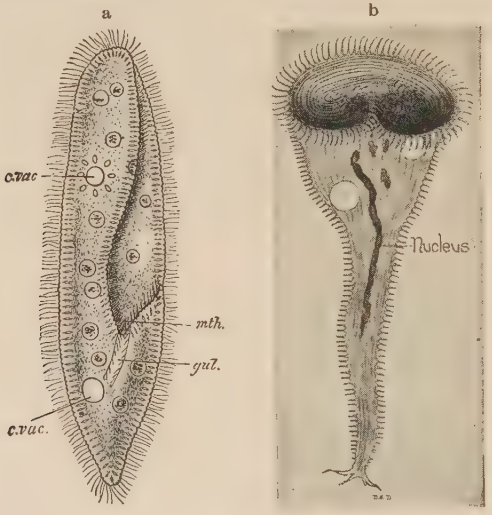

Fig. 38 a.—A one-celled animal, *Paramecium*, common in fresh water. The many cilia showing on this marginal view indicate how numerous they are on the surface as a whole. Such extensions aid not only in movement, but in absorption and elimination of soluble substances (food, oxygen and wastes, including carbon dioxide).

Fig. 38 b.—On the right, *Stentor*, with its curious necklace-like nucleus.

each cilium. (Fig. 38.) Many single-celled plants, including many bacteria, have similar extensions of the body allowing movement. (Fig. 66.)

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(See also general reference list in Appendix)

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REVIEW AND STUDY QUESTIONS

1. Name the parts of a cell and the characteristics of each.
2. Give at least two ways in which the character of the cell wall may affect the life or activities of the cell.
3. Contrast the nucleus and cytoplasm as important cell structures.
4. Show that the vacuole is an important cell structure in ordinary plant cells.
5. Distinguish at least three types of plastids, giving the characteristic functions of each.
6. List the cell activities common to all living cells and indicate for each one or more cell parts important in that work.
7. Illustrate the relationship between structure and function in a root hair cell, the chorophyll bearing cell of a leaf, or a water carrying tube.
8. Define osmosis, dialysis, plasmolysis, cell turgidity, cytoplasmic membrane, enzyme.
9. What parts of the cell have to do with reproduction? With securing food? With food storage? With cell movement?
10. Why is the ordinary green living plant called a more complex organism than yeasts or bacteria?

CHAPTER III

MOLDS (INCLUDING YEASTS)

LECTURE AND READING OUTLINE

A. The relation of molds to other plants, shown by the following classification of plants

(A) Flowering plants, reproducing by seeds (many-celled structures containing embryo plant)

(B) Flowerless plants reproducing by spores (single cells)

a. Ferns, well-developed stems, leaves and roots

b. Mosses, distinct stems and leaves; no true roots

c. Algæ, plants not differentiated into distinct stems, leaves, and roots. Range from single cells and filaments, to large plant bodies, as in giant seaweeds

(a) Red and brown seaweeds; chlorophyll present, but masked by red or brown pigment

(b) Green algæ (pond scum, thready growth on docks, at or below water level)

(c) Fission plants; most prominent group, bacteria; differing from all of above groups in never having sexual methods of reproduction. Cell division methods apparently simple (see mitosis, pp. 46, 47

d. Molds or fungi

(a) Belong with algæ, lacking differentiation into stems, leaves, etc. Differ from green or other colored algæ in lacking chlorophyll. Differ from bacteria and other fission plants in more complex form or structure and methods of spore formation. Sexual methods of reproduction common

B. Molds

(A) Fungi as degenerate plants

a. Simple in structure; but few types of cells in any one mold

b. These types but slightly differentiated from each other, even in aerial and absorbing threads (Fig. 39)

c. Chlorophyll lacking, molds therefore dependent

(B) Life history of molds
  a. Spore to mycelium to spore-bearing threads producing spores, which repeat the same cycle
    (a) Spores borne in a common spore case (Fig. 42)
    (b) Spores produced singly in sequence on spore-bearing threads (Fig. 43)
  b. Molds with double life histories, on alternating hosts
    (a) Note contrast in size, shape, color, length of life, period, in such double histories, as wheat and barberry rust (Figs. 52 and 53)

(C) Other prominent characteristics of molds
  a. Commonly two main parts: mycelium and spore-bearing threads
  b. Spore production prominently asexual; sexual method more common than formerly supposed, however
  c. Mycelial walls
    (a) Very thin, aiding absorption; often no differentiation in mycelium, *e.g.*, "roots," for absorption of water and food
    (b) Suited to moisture-holding habitats; note collapse of molds grown in laboratory when cover is removed; relate to aeration effects (breadbox, etc.) in eliminating molds
    (c) Water reservoirs large or very numerous, increasing with age, and causing less granular appearance of old threads
    (d) Rapid absorption of water, giving increase in size often confused with true growth
    (e) Water content major part (11/12 to 19/20) of weight; therefore small food value, even in larger molds (mushrooms), except as appetizers

(D) Economic relations

a. Helpful organisms

(a) General decay; shared with bacteria; necessary to return mineral constituents promptly to soil; related to depth of topsoil. Temperature relations favoring growth (including bacteria) important also in allowing plant utilization of many fertilizing substances (turned-in stubble, manure)

(b) Foods

1. Fungous growths themselves, such as mushrooms
2. Effects on other food substances: flavor, digestibility, as in cheeses (see milk products)

(c) As medicines, *e.g.,* ergot, a grain mold; extract used to check bleeding

(d) Commercial types, chiefly yeasts, usually without definite mycelium

1. Methods of reproduction in yeasts
   (1) Characteristic "budding" in vegetative growth (Fig. 36)
   (2) Spore production
   Conidia in many yeasts having mycelium
   If sporangia, few-spored, as in Figure 47
2. Yeast food materials
   (1) Protein or other nitrogen-supplying material essential for growth or formation of new cell substance; necessary minimum small, however
   (2) Carbohydrates, common sources of energy
   —Starches apparently utilized in bread making,

fermenting of grains, etc.; change of starch to sugar really due to accompanying organisms or to enzymes from plant cells supplying the starch (flour, corn, etc.)

—Sugar changes: two types of yeast enzymes

—Simple sugars change, forming carbon dioxide and alcohol: $C_6H_{12}O_6 \longrightarrow 2CO_2 + 2C_2H_5OH$. This work is done inside the yeast cells (zymase enzyme); heat and energy thus liberated are available to yeast cells

—Double sugars to simple sugars. Commercial yeasts use cane sugar (sucrose) and maltose, but not lactose. Sugars split by appropriate enzymes (sucrase and maltase) excreted from yeast cell into surrounding food material; simple sugars thus formed may pass into cell, where they are broken down by zymase enzymes, as described above

3. Conditions affecting growth
   (1) Organic foods necessary
   (2) Liquid or a moist environment essential, as in other molds
   (3) Temperature. Optimum, commercial forms, 23-40 degrees C.; for short periods much higher temperatures may be used. Lower temperatures with slower growth often used, thus allowing the development of bacteria yielding desirable by-products (flavor in breads)
   (4) Keeping conditions vary with type of yeast
      —Dry yeasts kept under moisture-free conditions, temperature unimportant; large percentage die, but molds, etc., do not multiply
      —Moist yeast (compressed yeast) kept at low temperatures, as moisture content favorable for growth of undesired molds and bacteria
   (5) Inhibiting agents
      —Hops in domestic liquid yeast or barm
      —lactic acid in commercial yeasts (beer)
4. Utilizable products. Mainly those of fermentation; gas or alcohol usually desired

(1) Gas in bread to lighten texture
(2) alcohol in wines and other liquors; liquors bottled before fermentation ceases often contain carbon dioxide as well as alcohol, giving the sparkle and "pop" of certain liquors
Alcohol held in solution in liquids; escapes from bread during baking. Vinegar from fruit juices has passed through a yeast-alcohol stage, after which acetic acid is formed from the alcohol by the yeast, or, more commonly, by other organisms. (See p. 205)

5. Types of commercial yeasts grown in liquid cultures, usually sold as
   (1) Dry cakes or nearly dry
   (2) Compressed masses of yeast with starch grains, corn meal, etc., to give bulk for forming cakes or loaves
   (3) Bakers' yeasts, sold in liquid form (pails, etc.)

6. Domestic forms
   (1) Barm or hop yeast
   (2) Wild yeasts
   Fermenting juices, sweet wines, etc., occasionally used in Southern Europe
   Salt-rising bread. Corn meal and milk are often used. Wild yeast and bacteria from meal and air cause fermentation; salt limits growth of undesirable bacteria. Other types named from source or from initial food material: potato water yeast, witch yeast (air forms), etc.

7. Yeast substitutes
   (1) Chemicals which form gas and cause porous condition
   (2) Mechanical aeration by beating or otherwise forcing air into dough; the flatness of flavor due to lack of usual by-products of yeast and associated bacteria

b. Harmful molds (yeasts included)
   (a) Human infections (often "secondary invaders")
      1. True yeasts
         (1) Not common
         (2) Infections: ulcers, "thrush," skin infections (blastomycosis)
         (3) Mosquito-bite theory: initial swelling possibly due to yeasts injected by plant-feeding insects; such yeasts have possibly symbiotic relationship in insect intestine: yeasts gain food and also aid insect in partly digesting the food and inhibiting intestinal bacteria
      2. Other molds
         (1) More common than infections by true yeasts
         (2) Infections
            —Skin: ring worm, favus, pityriasis
            —Throat: chronic sore throat
            —Internal tissues (Fig. 45, II): abscesses and tumors; often due to intermediate mold-bacteria group (Actinomyces or Nocardia)
   (b) Other animals
      1. Cow: "lumpy jaw"; lung infections
      2. Fish: water molds, readily penetrate gills and egg sac while still attached to young fish; loss in hatcheries heavy

(c) Molds causing other economic losses

1. Foodstuffs. Decay or spoilage in homes, factories, stores, and during transportation and storage
2. Plants of economic importance: food-producing plants (potatoes, beans, melons); textile plants (cotton); lumber (white pine)

(E) Yeast study as an introduction to bacteriology

a. Involution forms common, especially in old cultures; relate to difficulty of diagnosis in bacterial infections, such as diphtheria
b. Rapid growth and fermentation, allowing the production and identification of products (CO_2) in single class periods
c. Safer than most gas-forming bacteria for experimental work by beginner, and therefore useful as introduction to diagnostic work (kind of gas, per cent. of given gas)
d. The relation to environment changes (food, temperature) easily shown
e. Enzyme activities easily illustrated (see p. 53)
f. Size favorable for preliminary staining exercises
g. Invisible nucleus may be demonstrated by ordinary bacteriological stains

DEMONSTRATION AND LABORATORY WORK

1. Making a mold culture.*

Place a sterile cracker in a sterile Petri dish, as shown by the instructor. Moisten this cracker with sterile water (cotton-plugged test tubes of water previously sterilized with steam), adding only as much water as the cracker can soak up. (Too much water favors bacterial rather than mold growth.) Then with sterile forceps inoculate the moist cracker with a tiny bit of mold selected from one of the dishes of molds on the supply table. Label, giving the date and the type of mold with which the cracker was inoculated. Leave under conditions (temperature, darkness) you think most likely to give you a good growth by the next session of the class.

Compare your results with those obtained by the rest of the class to see if the differences can be explained as due to (1) type of mold, (2) apparent purity or impurity of the culture, and (3) the relation of water, light, and temperature to the rate of growth.

*This exercise should be set up about three days before the class is ready for the rest of this section. The instructor will find it advisable to make daily Petri dish cultures of the types of molds to be used for study, beginning about five or six days before the molds are needed.

2. Examine these dishes of mold with a hand lens, and note the general cobwebby character of the mycelium. The large bulk, compared with the small amount of food apparently used from the cracker, will be explained if the molds are examined under the microscope and the large water content of the threads seen.

3. With the forceps pick up one little cluster of mold (Fig. 39) from the upper cover of the Petri dish provided by the instructor, which contains the ordinary black mold. Place it

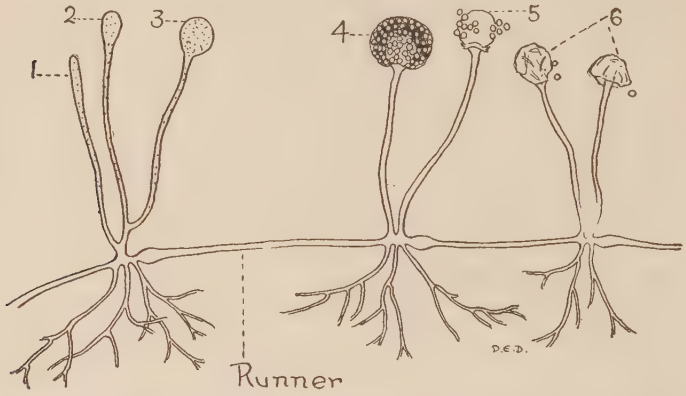

Fig. 39.—Ordinary "black mold" or "bread mold." The numbers indicate progressive stages in the formation of the sporangia and the ripening and distribution of the spores.

promptly in a drop of water on a slide and cover gently with a cover-glass to avoid crushing the delicate threads. (Be prepared for a large number of bubbles, Fig. 40, ranging from small black rings or iridescent masses to large irregular black areas following the main stalks of the mold, because the surface of the mold is often very oily and does not readily become wet with water, which allows air to remain between the thread and the water).

Note the threads or runners connecting these little plants in Figure 39. Find on your plant evidence that such runners carried food from the cracker to the plants on the glass cover. Locate the root-like parts which serve (1) to hold the plant in position, and (2) as absorbing organs (food and water).

Draw the mold plant as you see it, labelling (1) the absorbing part of the mycelium (2) the spore-bearing threads, and (3) the

spore case with its enclosed spores. Search slides made by your fellow students as well as your own to see if you can locate some of the younger stages of the spore case shown in Figure 39.

Find young threads apparently filled with granular contents, and older threads with large round or elliptical water reservoirs

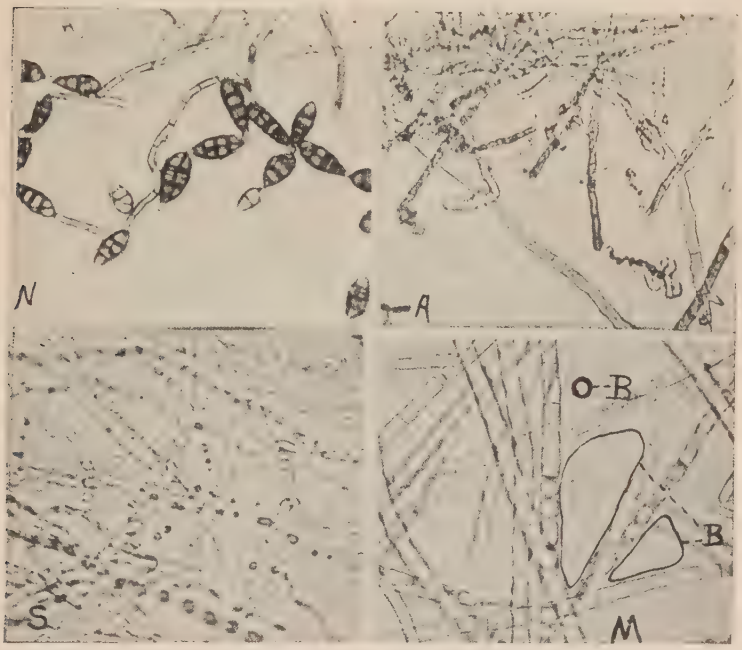

FIG. 40.—Altenaria. N, normal growth, with compound spores (see Fig. 52); M, older growth showing threads with large water reservoirs; S, very abnormal growth with numerous water reservoirs in 50 per cent. sugar; and A, stunting and disintegration in .04 per cent. acetic acid. (Two types of air bubbles are present (13), Section M. Pressing upon the slide while examining it through the microscope will usually enable you to decide whether you are looking at bubbles or mold structures.) (Reduced from Bitting: The Effect of Certain Agents on the Development of Some Molds.)

or even continuous water channels throughout the threads. Do you see any cross walls or other indication of cell structure, as in Figure 43?

4. Leaf through the text-books on the demonstration table until you find among the molds pictured there illustrations of the following: (1) mycelium *with* cross walls, (2) without specially developed attachment of food-absorbing threads or

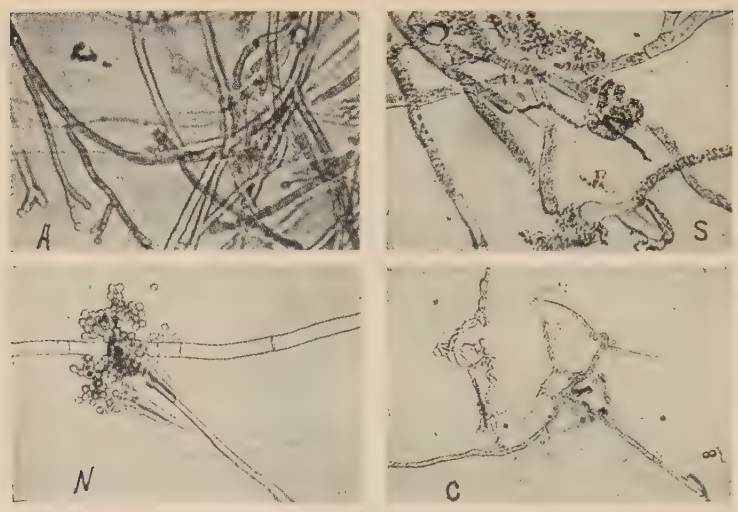

FIG. 41.—Penicillium. N, normal; stunting and disintegration are evident in the other sections: A, grown in 1 per cent. allspice; S, in 0.1 per cent. sodium benzoate; and C, 1 per cent. copper sulphate. (Reduced from Bitting: The Effect of Certain Agents on the Development of Some Molds.)

FIG. 42.—Figs. 39 and 43 show the two main types of spore production: in a common spore case (39) and by successive constriction or cutting off of spores or conidia (43). Three types of conidia are shown in this illustration. In H (*Spoerothecia* on hops) spores are produced much as in Fig. 43; in L (*Bremia* on lettuce) the conidia are produced in fours on very branched stalks; in A note the compactly clustered spores, some of which are germinating (*Pleospora* on alfalfa). (After Schwarze: Bulletin, N. J. Agricultural Experiment Station.)

"roots" and (3) spores *not* in a common spore case, but singly, or in lines or clusters at the ends of the mycelial threads.

5. Select next for study one of the red or blue (green) molds from the Petri dishes on the supply table. Mount and examine small masses of the mold until you can reconstruct what you

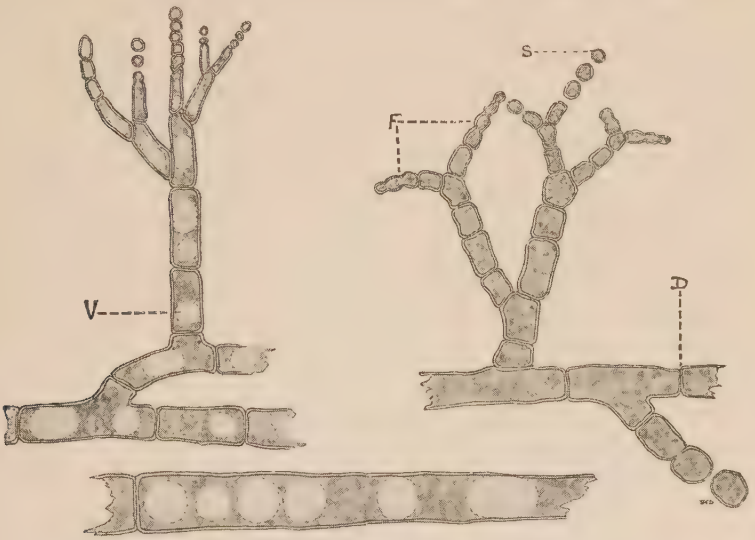

FIG. 43.—A septate mold showing definite walls or septa (D) in the threads or hyphæ. Note at F the incomplete constriction of the tips into spores; complete division shows at S. Distinct water reservoirs (V) are present here.

think is a typical mold plant of this kind. What differences do you observe between the myceliums of the two molds studied? In the method of bearing spores?

6. With the instructor use the key for identifying molds in one of the books provided (Buchanan, Tanner) to trace one of the molds studied in class. Write the scientific name of the mold under the appropriate drawing. Then trace the other mold by yourself, verifying your results by consulting the instructor.

7. In the molds studied, the nuclei were not visible under the microscope. These specially-stained threads (Fig. 44) show one or more nuclear bodies in each division of the mycelium.

8. Place a drop of growing yeast from the molasses and water

mixture provided (three teaspoonfuls of molasses to a glass) on a slide, and examine under the microscope. A little iodine will

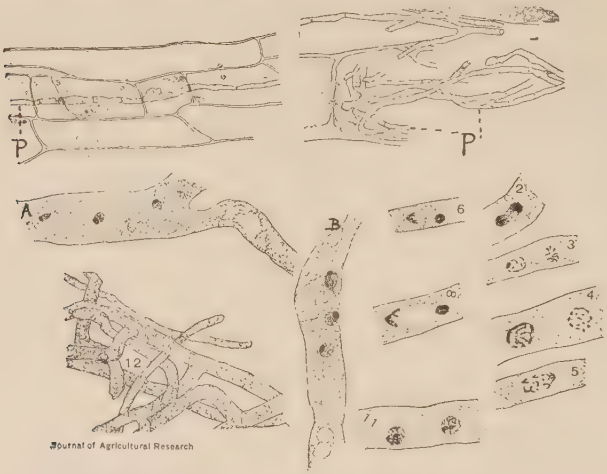

Fig. 44.—Mold threads (*Phoma* in beets) penetrating leaf cells to obtain food are shown at P. Consult Fig. 52 to find another method used by parasitic fungi. That mold threads, such as those seen in Fig. 39, are really composed of many distinct cells is shown by such specially stained preparations as A and B in the above illustration (*Rheosporangium*). Parts 2 to 11 show stages in nuclear division very like the mitosis changes seen in higher plants (Fig. 35). (Both reduced from Edson, Jour. of Agr. Research).

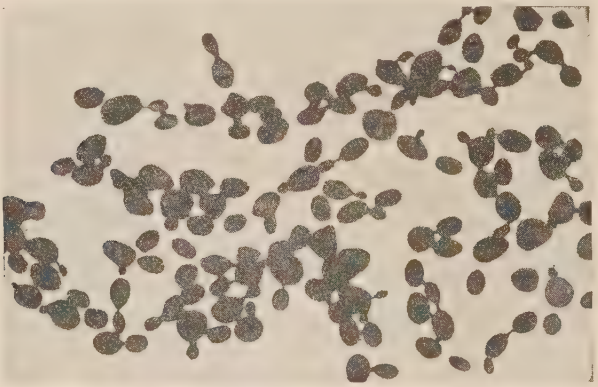

Fig. 45.—Budding bread yeasts. What is the largest number of cells still adhering to the parent cell?

stain the cell substance a yellow-brown, and make it easier to distinguish the large vacuole which almost fills the cell (Fig. 45).

Locate one or two of the accompanying starch grains (by their iodine reaction), so as to gain a better idea of the relative size of the small yeast cells.

9. Draw one yeast cell on a larger scale making it appear about half or three-fourths of an inch in diameter. Label the cell wall, cytoplasm and the vacuole. Nuclei are denser masses of material and might be expected to appear darker at various focal levels. Can you distinguish any nuclei in these iodine-stained yeasts? Is this true of the yeast (Fig. 46) cells specially stained (methylene blue)?

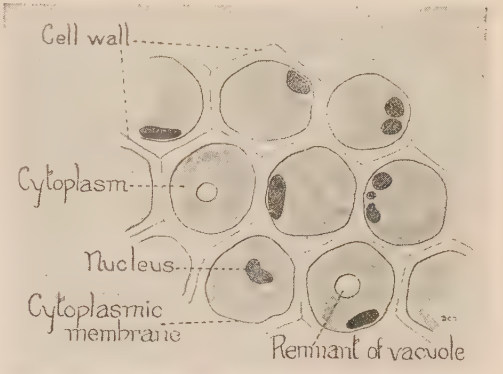

Fig. 46.—What three changes are indicated in these stained yeast cells? (See Exercise 10).

10. Spread a drop of yeast and water out in a thin smear on a glass slide; dry it and stain it with methylene blue, a bacteriological stain to be used often in the following part of this course. (See p. 115.) After staining, add a drop of water and cover-glass, as shown by the instructor, and examine under the high and low power, studying especially the colored masses showing organisms lying close together (Fig. 46). How have these stained cells changed with regard to their cell outlines? Are the same cell parts visible? What changes with regard to nucleus and vacuole? What effect has this staining process (drying, heating, the alcoholic content of the stain) upon the water content of the yeast cells? Show that this loss of water explains all three changes observed in these stained cells. Do these changes show that the staining processes may be important in diagnosing disease (throat smears for diphtheria)?

11. Examining materials. With a sterile toothpick rub a little soft cheese (cream or cottage) on a clean glass slide. Dry

and then wash in alcohol for one to two minutes, as shown by the instructor, to fix the organisms to the slide. Drain dry and then wash in xylol, as shown, one-half to one minute; this removes most of the fat, some of which was previously removed by the alcohol. Drain dry and then stain as usual with methylene blue (p. 115). This method fixes the organisms to the slide. We use alcohol instead of heat as heat tends to "cook" the milk, making opaque or shreddy masses over the surface of the slide.

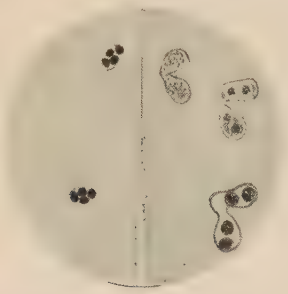

Fig. 47.—Spores in ordinary yeasts are usually in threes or fours. On the right is a drawing of the rather unusual zygospore (sexual) stage found in some yeasts, in which the cells fuse in pairs before the spores are formed.

Add a drop of sterile water and a cover-glass and examine to see if the yeasts present show nuclei more distinctly than do the ordinary bread yeasts. Mold threads which show nuclear bodies are often present in cheeses.

Look also for bacteria, rod or coccus forms, so as to gain an idea of the relative size of the bacteria. Indicate roughly this difference in size by a drawing in the margin. (This method of examining material is not essentially different from the method of examining food material or pus, or other pathological substances.

12. Spore production in ordinary yeasts (Fig. 47). Note the few spores in each cell compared with other molds.

13. Yeasts from an old molasses culture. Draw at least two unusual shapes (involution forms) from those shown in the microscope (Fig. 48).

14. Yeasts from a sour milk preparation (Fig. 49). The yeasts were originally obtained from ordinary compressed yeast, like those shown above, Exercise 8, Figure 45. Note the tendency to form a mycelium under these conditions.

15. Use one of the classifications cited at the end of this chapter to find the name of the yeast you have been studying (Exercises 8 to 10 above).

16. Experiments with yeasts to show characteristic cell activities.

1. Is carbon dioxide formed by yeasts?
2. How much (what per cent.) of the gas formed by yeasts is carbon dioxide?
3. Does cell activity depend upon the type of food material provided? For this, use dextrose (or glucose), molasses, cane sugar, starch, and flour.

FIG. 48.—Involution forms in an old yeast culture.

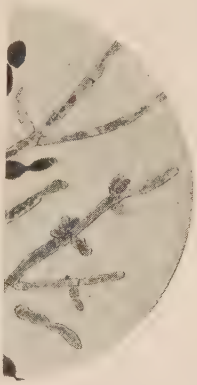

FIG. 49.—Yeasts differ in the rate at which they tend to develop the mycelial form. This is an extreme stage rather readily shown by ordinary compressed yeast in acidophilus milk cultures.

4. Show that temperatures affect the activity of yeasts (convenient contrasting temperatures are melting ice, room temperature, 37° to 45° C., and boiling temperature).
5. From experiment 4 select the tubes showing little or no growth and demonstrate whether or not the temperatures used have killed the cells or merely inhibited the cell activities.

(These experiments might advantageously be done by setting them up one at a time at the beginning of a class period. When the course covers but a short interval it would be better to have the class do them all in a given day, though naturally one experiment is the outgrowth of another; for example, having shown that yeast gives off CO_2, the

student naturally asks, "Is all of that gas CO_2"? or "What per cent. of the gas is CO_2"? If done as a general class exercise the best scheme is probably to have the students discuss the method of performing each experiment, and assign the several experiments to small groups of students. At the next class session the experiments might be set up as soon as the students enter the room, and the results obtained by the various groups shared with the rest of the class).

(a) Make out a complete list of the materials you will need for the experiment assigned to you or your group of students, so that the

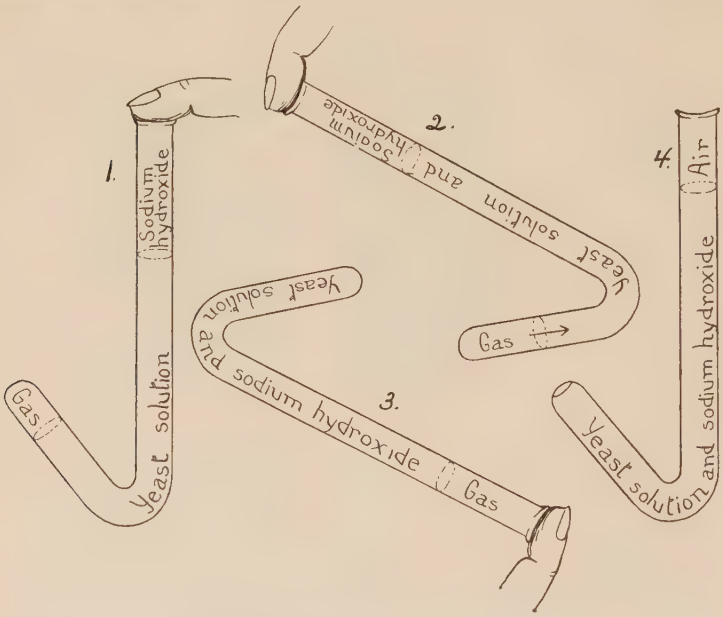

FIG. 50.—See Exercise 16 (c). 2. Number 1 shows the tube after sodium hydroxide was added; number 4 shows the condition after the sodium and gas were thoroughly mixed and the finger was removed.

materials may be in readiness for you at the beginning of the next period.

(b) Set up your experiment as quickly as possible, that results may be obtained in the class period. Record the exact time when the experiment is set up. Note the first change that occurs, recording the time interval. Call the attention of the rest of the class to such changes as they occur, that they may share fully the benefits of your experiment.

(c) Helpful hints

1. Lime water (or barium water) may be used in experiment 1.

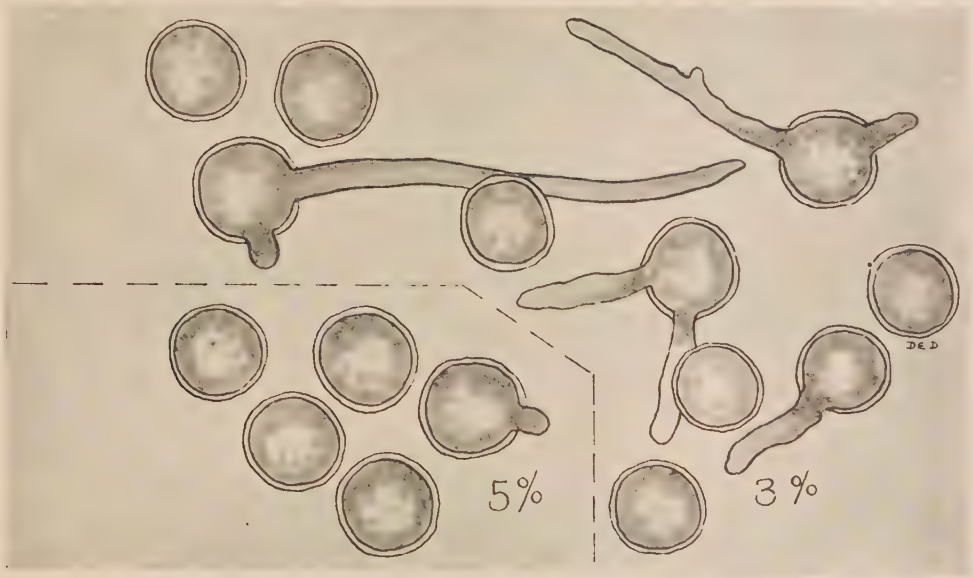

FIG. 51.—Mold spores from 3 per cent. and 5 per cent. sugar are shown above. No germination was noted in distilled water; very few had germinated in the 10 per cent. sugar. How can we explain all these differences?

2. In experiment 2, sodium hydroxide (3 to 5 per cent. solution) may be used to absorb any CO_2 present in the gas; add for the gas collected at least an equal amount of sodium hydroxide, filling the open end of the tube completely. Close with the finger and mix thoroughly (by tilting the tube, Fig. 50), and, after collecting the gas at the closed end of the tube, remove the finger. Why does the liquid move up into the closed arm? How much of the former gas-filled space does the liquid now occupy. How much of the gas was absorbed? How much of the gas given out by yeasts is CO_2?
3. The rate at which food is utilized is of course indicated by the rate of gas formation: the rate at which the bubbles rise in the liquid containing the growing yeasts. How may we make sure (type of vessel, height, and diameter) that these bubbles will be clearly visible?

17. Mold spores (Fig. 51), left for several days in sugar solutions of the strengths indicated. How can we explain the lack of growth in the distilled water? In the ten per cent. sugar solution?

18. Some molds can grow in very strong solutions of sugars or salts. This bottle shows a mold which developed "by chance" in a ten per cent. solution of salt made up for the plasmolysis experiment with the onion cell.

19. Find, in your home experiences, another instance where a particular mold has shown its power or ability to grow in high concentrations of sugar, salt, etc., such as mold in the salt brine of pickles, or "rust" on mackerel kept in brine.

20. Look through the advanced texts on bacteriology and find two instances of molds causing human infection.

Find one mold which grows on the scalp. What cautions should be observed regarding public toilet appliances (combs and brushes) and exchanging hats (children's games)?

21. The barberry rust, our best known example of a mold having two alternating hosts, is shown here. The first illustration (Fig. 52) shows the appearance on the barberry leaf and the structure of the spore-bearing part of that phase. The second illustration (Fig. 53) shows the external appearance of this mold on leaves of wheat and oats with the two types of spores found in this stage. There are molds which have three alternating hosts instead of two. About one hundred such molds with two or more hosts have been described.

22. Later, find an instance in which bacteria or protozoa live similarly in alternate hosts. Wheat may be protected by eradicating the native barberry plants. Find two bacterial or protozoan

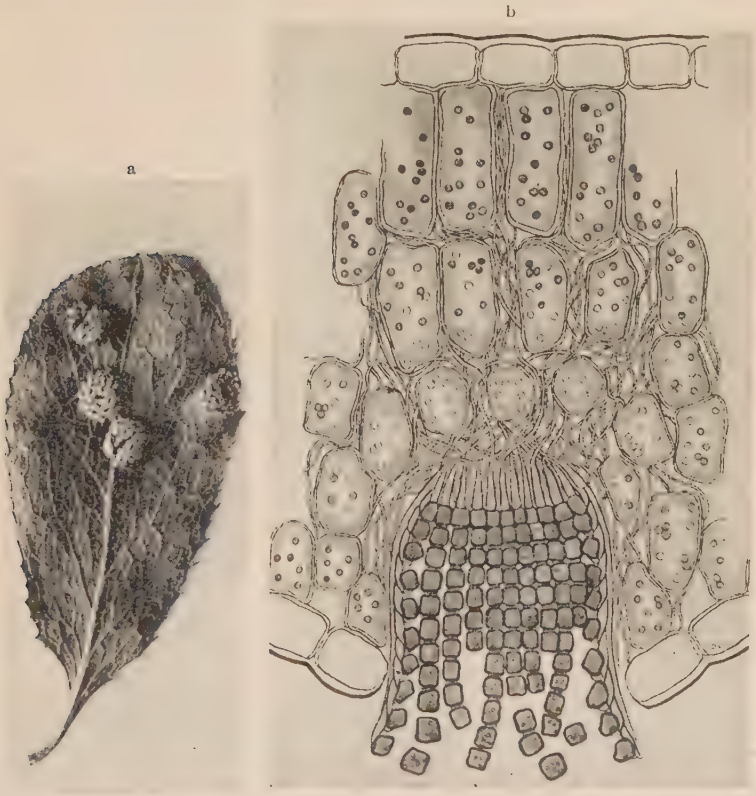

Fig. 52 a.—A barberry leaf showing massed fruiting bodies or "cluster cups." (Howitt, Bulletin, Ontario Agricultural College.)
b. A section through a single cluster cup is shown enlarged. Note also the position of the mycelial threads between the leaf cells, thus interfering with the ordinary transfer of materials from cell to cell.

diseases in which we protect man by eradicating the alternate host. (See Exercise 2, p. 80.)

23. Look through the collection of pamphlets and books listed at the end of the chapter which picture mold parasites on plants, including trees of economic importance. Find one

disease affecting a plant valuable in your community and give a brief abstract describing the disease, its effects, and methods of control.

Find one plant disease in which control is best obtained *indirectly,* by selecting or breeding resistant strains or types of plants (see Frontispiece). Later, find for animal diseases a similar situation.

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(See also general reference list in Appendix.)

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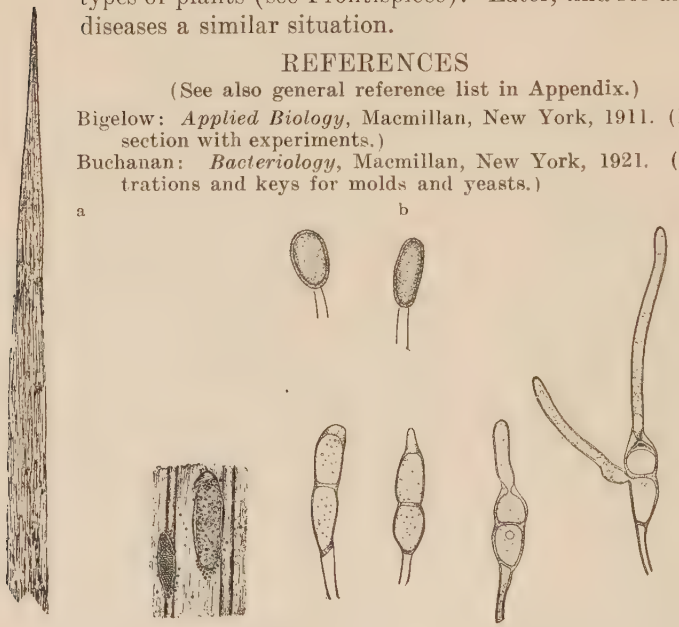

FIG. 53 a.—Part of mold-infected leaf of wheat, with an enlargement (at the right), showing two areas where the spore-bearing tips of the mold (*Puccinia*) have broken through the epidermis, giving the streaked rusty appearance seen in infected leaves and stems of wheat, oats, etc.

b. In this are shown two types of spores produced by such rusts; the ones pictured here are the single reddish-brown spores and the double dark-brown spores found on cultivated asparagus. Germination of such double spores is shown on the extreme right. (After Schwarze, Bulletin, N. J. Agricultural Experiment Station.)

Duggar: *Fungous Diseases of Plants*, Ginn, Boston, 1909.

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(For other pamphlet references see Chapter XI)

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REVIEW AND STUDY QUESTIONS

1. Give the general structure and life history of a common mold.
2. Describe two molds which differ markedly in structure and in simplicity or complexity of their life history.
3. How do yeasts and other molds differ from ordinary green plants?
4. Why are yeasts rightly classed as molds?
5. Name for molds two different types of myceliums; two differences in spore production; two differences observed in the spores themselves.
6. Picture what would happen in our world if all mold activity ceased to-day.
7. Sum up the facts gained from the five experiments with yeasts so as to furnish directions for:
   (1) keeping compressed yeast cakes
   (2) obtaining rigorous yeast for starters
   (3) giving the best results in baking.
8. Illustrate what is meant by "alternation of hosts" in fungi.

CHAPTER IV

BACTERIA (AND PROTOZOA)

LECTURE AND READING OUTLINE

THROUGHOUT this course the emphasis is upon bacteria rather than protozoa. This seems wise for the following reasons:

a. Bacteria are more important in economic processes and relationships than protozoa
b. In this latitude more of the human diseases are due to bacteria than to protozoa
c. Protozoa are more difficult to cultivate on ordinary laboratory media than bacteria are
d. In general, the statements made concerning bacteria (their vital phenomena, the effects of physical and chemical agents, the reactions of the body, etc.) hold for protozoa also

A. Relative position of bacteria and protozoa in biological classifications

(A) Each the simplest of lowest group; bacteria in plants (see p. 50); protozoa in animals

(B) Bacteria and protozoa; not always easily distinguished from each other

a. Likenesses
   (a) Both small, usually microscopic; same descriptive terms (microbe, micro-organism) applied to both
   (b) Both non-green, dependent organisms, wholly or in part dependent upon organic foods (exceptions very rare, not found in micro-organisms discussed in this text)

b. Differences
   (a) Protozoa usually larger than bacteria, though some are ultra-microscopic, and can pass through even the best types of filters
   (b) Protozoan cells are more flexuous
   (c) Differences in cell parts
      1. Wall present in bacteria; body of protozoa less rigid
      2. Vacuole more prominent in some protozoa
      3. Nucleus distinct from cytoplasm in protozoa
      4. The life history is often more complex: many protozoa pass through a definite life cycle

(d) Staining qualities
1. Wider range of affinity in protozoa
2. Related to wider use of drugs and stains in treating protozoan diseases, such as sleeping sickness, and malaria; less often helpful in bacterial infections, though occasionally useful (wounds)

B. Bacteria

(A) Distribution of bacteria
a. Almost universal presence indicated
(a) By wide range of habitats: water, soil, milk and other foods, sewage, skin and other tissues (mouth and nasal membranes)
(b) Wide-spread evidences of fermentation and putrefaction

(B) Difference between habitat and temporary or accidental presence related to
a. Conditions such as food and moisture, favoring growth (mouth, habitat of staphylococcus; skin, more temporary presence)
b. Vigor and virility of the organism
(a) Resistance to untoward conditions
(b) Invasive power, as in body tissues

(C) Morphological characteristics
a. Size and weight
(a) Minute; compare cells already studied: onion cell, yeast
(b) Unit of measurement used: micron = 1/25,000 inch
(c) Range in size of well-known forms
1. Large water bacteria: 25–60 microns; determine in inches length of bacterium 50 microns long
2. Smallest known forms, apparently pathogenic
(1) Shortest known: influenza, 1/2 to 1/10 micron; infantile paralysis "globoid" bodies, 1/10 to 3/10 micron in diameter

(2) Ultra-microscopic organisms; their presence proven by infectious quality of material which has passed through best known filter and lacks visible organisms; imperceptible highest known power of microscopes (foot-and-mouth disease?), hog cholera

3. Average bacilli about 2 1/2 microns; average coccus forms, 8/10 to 1 micron in diameter

(d) Comparison of size and weight

1. Weight of individual cell determined by weighing a mass and calculating number of organisms present in a definite unit or part of that mass spread out on a glass slide
2. Weight of average bacterial cell calculated as 2 billionths of one milligram. Estimate number of bacteria possible in one ounce of milk, should ¼ of the weight be bacteria and ¾ of the weight milk substance. How does this estimate compare with the highest count you have seen recorded for milk?

b. Cell parts

Terminology of various standard authors confusing because such terms as cell wall, ectoplasm, and capsule are not really interchangeable. In terms of ordinary plant cells, we may consider all bacteria as possessing a definite cell wall, inside of which are contained the cytoplasm (endoplasm) and its enveloping cytoplasmic membrane (ectoplasm). Bacteria may or may not have a capsule outside the cell wall

(a) Cell wall

1. Distinct membrane giving fixed shape to cell; compare changing shape of protozoan cell (Fig. 99, II)
2. Incidental differences: presence of spores causing swollen ends (tetanus) or bulging of central part of cell (gas gangrene)

(b) Capsule

1. Staining qualities: differs in affinity for or resistance to stains as indicated by difficulty in staining (tuberculosis organisms), or loss of stain by capsule on bleaching (acid, alcohol) in such staining methods as Gram stain and acid-fast stains
2. Presence of capsule may vary with food (Fig. 40, II); with age
3. Possible relation to resistance of cell to injurious agents, as resistance of capsulated individuals (*Streptococcus*) to white corpuscle destruction

(c) Flagella

1. Protoplasmic projections of cytoplasmic substances giving motility to cell with power of varying environment: presence of food; unfavorable conditions (light, oxygen relations, waste substances)
2. Rate of movement magnified by microscope; determined rate for cholera, 18 cm. per hour; compute in terms of own average length, 1.5 microns
3. Type of movement more or less related to number and arrangement of flagella, often quite characteristic: rapid, darting, sinuous, rotary, jerky, etc.

(d) Cytoplasm

1. Rarely to be distinguished from included nuclear material (see nucleus below). Distinct in certain large forms, as water bacteria (Fig. 69)
2. Food granules (starch, fat, glycogen) distinguished from nuclear granules or nucleus and from each other by staining properties (fat shown by osmic acid)

(e) Nucleus. Presence assured by
1. Chromatin, a constant constituent of nuclei
   (1) Nucleus sometimes described as represented by chromatin scattered throughout cell
   (2) Whole cell may be considered as mainly a nucleus; (compare similar conditions in sperm cells of animals)
2. Type of stain: basic or nuclear stains used for bacterial stains; acid stains are used for general cytoplasm stains
3. Further proof of presence of nucleus obtained by chemical analysis: dried tuberculosis organisms show nearly 60 per cent. to be nuclear substances (nucleic acid, etc.)

(f) Pigment
1. Not in definite plastids, as in higher plants
2. Distinct from such color substance as sulphur granules
3. Often due to oxidation changes in excreted or waste substances; compare pigment present in bacterial masses with and without contact with oxygen (Fig. 84)

c. Cell division
(a) A nuclear function, as in all cells
(b) Division line may be related to position of metachromatic (nuclear) granules. (Fig. 61.) In a few carefully studied forms, division of such granules precedes division of whole cell
(c) Time required for full development of two new cells from one cell may be but 20–30 minutes. Compute possible number developing from one organism in six hours;

in one day. In how short a period might a million organisms be developed from a single cell?

(d) Multiplication rate affected by environmental conditions

1. Food
2. Water supply
3. Accumulated waste, results of cell metabolism; illustrated by death of organisms in old cultures while food still present
4. Temperature; direct effect upon rate of growth; effect seen in spoilage time (milk)

d. Spore production; an adaptive response to unfavorable growth conditions (heat, lack of moisture, accumulation of waste substances)

(a) Number limited, one per cell in true bacteria; compare yeasts and other molds, also protozoa. Result is reproduction without multiplication of number of organisms

(b) Limited to few types of groups of true bacteria: *Bacillus, Clostridium;* occurs in some spiral forms; not yet unquestionably demonstrated in coccus forms

(c) Characteristics of spores

1. Refractile, especially when unstained
2. Resistant to stains

(1) Spore stains involve the use of heat or other mordants

(2) Once stained, the stain is retained so that bleaching substances (acid, alcohol) make it possible to remove the stain from the cell wall and remnant of cell substance without affecting the spore

(3) By using a contrasting counterstain a spore of one color may be shown enveloped by a differently colored or stained envelope

(d) Relation to human disease

1. Few human pathogenic bacteria produce spores: anthrax, tetanus, malignant edema and gas gangrene organisms develop spores
2. Lack of wide distribution of such organisms allows pasteurization of milk at relatively low temperatures; also the disinfection of infectious material (clothing, dishes, etc.) with weaker disinfectants than would be otherwise possible

e. Chemical composition

(a) Living cell: the cell substance of bacteria is generally considered very complex despite the simple morphological character of these cells. The types of organic substances present are varied and numerous. Water makes the preponderant part, as in most plant cells; 77-88 per cent. in bacterial cells

(b) Analysis of dry cell substance

1. Wall, mainly protein, contains various substances: hemicellulose (tuberculosis)
2. Living cell substance (cytoplasm and nuclear material). Various albuminous substances represented with fats, glycogen, nuclein, and nuclein bases or compounds
3. Inorganic substances, mineral matter, making 1 to 8 per cent., sometimes more, of the dry cell substance. Specific minerals (sulphur, ferric oxide) prominent in certain species (sulphur bacteria, iron bacteria). Minerals commonly present include
   (1) Such salts as salts of potassium, phosphorus, chlorine, calcium, sulphur, iron and silica
   (2) Phosphoric acid in large amounts; often one-half of total dry weight

f. Classification of bacteria

(a) Group or generic differences of early classifications

1. Based on morphological differences (Fig. 66)

(1) Shape of individual cells (spherical, rod-like, spiral). Cocci (sing. coccus), include about 300 named species. Bacteria and bacilli (sing. bacterium and bacillus), about 800 named species. Spirilla (sing. spirillum), about 100 named species.

(2) Relative arrangement of cells (pairs, distinct chains, cubes)

(3) Number and position of cilia or flagella (single, grouped, terminal, distributed over a whole surface)

2. Difficulty in identifying by early descriptions

(1) Shape and size alone not distinctive enough; variations in size too slight in large groups, such as rod-like bacteria or bacilli, which contain hundreds of species

(2) Presence of mutation and involution forms; variations as great as in yeasts (Fig. 48)

(3) Flagella inconstant or demonstration difficult in many species; special staining methods necessary

(b) Addition of physiological to morphological characters in newer classifications

1. Generic characters: such physiological activities as production of pigment, the formation of acids or alkalies are used to form a larger number of small groups of organisms, such as *Erythrobacterium,* now *Serratia,* for bacteria and *Lactobacillus* for bacilli forming acid from lactose. (Other examples given in classification in Appendix)

2. Specific differences

(1) Mainly physiological; illustrated in the genus *Streptococcus* by such differences as those shown when grown in blood agar: *Str. viridans,* the presence of green pigment; and *Str. pyogenes,* the dissolving effect on red blood cells

(2) Morphological characters, less striking or distinctive than the generic characters (capsule variations; the number of organisms adhering in a chain) (See Fig. 147)

DEMONSTRATION AND LABORATORY WORK

1. Ameba and bacteria (Fig. 54), affording a contrast between two types of one-celled organisms. Find in this and the following illustrations the contrasts noted on page 72.

FIG. 54.—Ameba with ingested bacteria to allow a contrast in size as well as in other points referred to in Exercise 1.

2. Many other animal parasites, higher types than protozoa, are the cause of disease. These include round worms (trichina, hookworm), flat worms (tapeworm), etc.

(a) Look through these general text-books (Stitt, Jordan, Park, Moore) and find one disease due to a small parasitic animal not a protozoan.

(b) Find two diseases due to very different types of protozoa.

(c) Find two illustrations or titles which indicate a definite life cycle.

(d) Find two protozoa which need alternate hosts for their complete development.

FIG. 55.—This illustration shows an early stage in the fusion of the nuclei in these two protozoan cells, *Paramecium*. Bacteria differ from most other plants as well as animals in having no sexual reproductive phase, depending upon fusion of two cells of nuclei.

3. These texts (Moore's *Microbiology,* Calkins' *Protozoölogy*) give a simple classification of protozoa. The groups containing the species of special interest to us have been marked by the instructor. Most protozoa, you will note, are not pathogenic to man and the important domestic animals.

4. Look through Protozoan Parasites of Domesticated Animals, by Crawley (Circ. 194, Bureau An. Ind., 1912). Are any of these also human parasites?

5. Paramecium (Fig. 55), another of the protozoa. Note the large size and the distinct nucleus. These cells are conjugating; bacteria have no sexual phase like this.

6. Trypanosomes from the blood of a rat (Fig. 8, II). Note the distinct nucleus and the very flexuous body. Which are more numerous in this blood sample, the blood corpuscles or the parasitic trypanosomes? Note the curious "fold" or ridge running

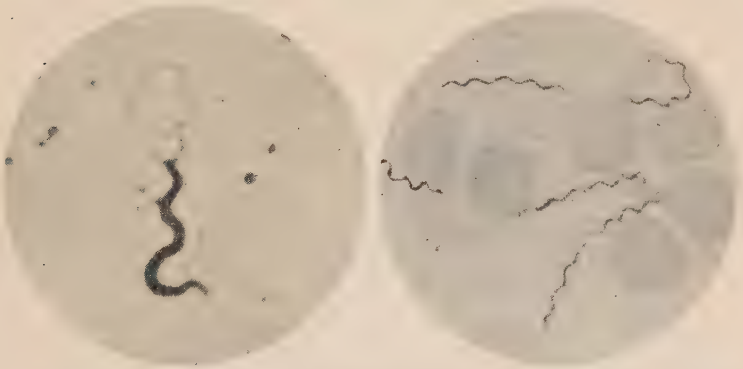

FIG. 56.—A spiral protozoan, *Spirillum obermeieri*, the cause of relapsing fever. The one on the left is more highly magnified and shows a terminal flagellum. (Novy and Knapp, Journal of Infectious Diseases.)

lengthwise of the body and the flagella. Trypanosomes are more common causes of diseases in the lower animals than in man.

7. The spirochæte of relapsing fever was the first one definitely related to disease (Fig. 56).

8. Another spirochæte, *Treponema pallidum* (Fig. 115, II) the cause of syphilis; spiral protozoa have more turns or spiral curves than the spiral bacteria (Fig. 108, II).

9. Coccidium, a little protozoan that causes various diseases in animals. This slide was made from a drop of bile from a rabbit having a heavy infection, the liver and lungs appearing heavily dotted even to the naked eye with masses of these ellipsoidal organisms (Fig. 57).

10. In malaria the alternate hosts, mosquito and man, are less

intimately associated than are the hosts of the Texas fever parasite, where the tick attaches itself to the cow. The life cycle

Fig. 57.—Life cycle of *Coccidium*. The small forms entering the epithelial cells (as in 5), multiply to form similar bodies (leaving in 7a). This phase may be repeated over and over. At times apparently similar stages (7a) develop into two different forms (sperm cells 8a to 10a, and egg cells 8 to 11) between which fusion occurs (11). This sexual stage produces in turn the original form of the organism (4, 4a). Which stage is shown in the accompanying microscope? (After Schaudinn.)

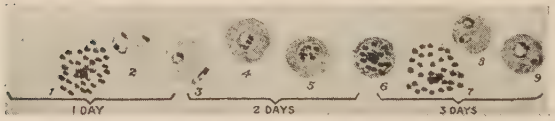

Fig. 58.—Malaria organisms affecting human red corpuscles (2 to 4). These organisms at intervals divide into spore-like bodies (5, 6) which are liberated into the blood stream (1, 7) and attack new blood corpuscles (8, 9). Figures 42 and 43 II correspond to 2 and 3 in this illustration. (Park, Williams and Krumwiede: Pathogenic Micro-organisms, Lea and Febiger.)

of the malaria organism is apparently much more complex. See the accompanying diagrams (Figs. 58 and 59). Which stage is shown in the blood slide under the next microscope (See Figs. 42–43, II)?

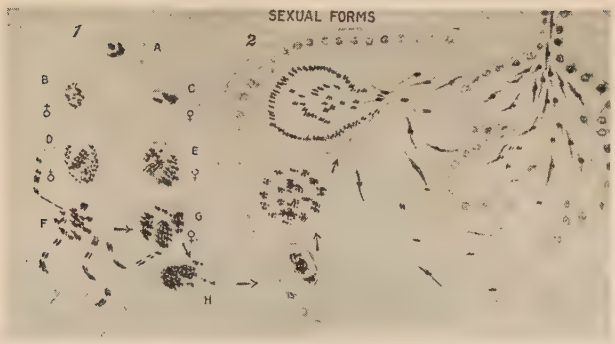

FIG. 59.—When the mosquito bites human beings affected with malaria, the organisms may be sucked in with the blood. In the mosquito are developed types F (male cells), and O (female cells), which fuse in pairs. In the walls of the stomach these paired cells develop into spindle-shaped bodies which finally (10 to 14 days) find their way to the salivary glands, where they may persist throughout the life of the mosquito, thus giving a long danger period. (Park, Williams and Krumwiede: Pathogenic Micro-organisms, Lea and Febiger.)

11. Mashed bit of brain tissue, showing Negri bodies (Fig. 118, II). Study the labelled illustration, and identify red blood corpuscles, nuclei of disintegrated nerve cells, and Negri bodies, now accepted as the cause of rabies.

12. Size and shape alone are more helpful in distinguishing the protozoa from each other than they are in differentiating bacteria. For bacteria, we commonly use also cultural characters (broth, gelatin, and agar). In the subsequent demonstrations, find one instance where one or more of the following characters seems of value: pigment production, surface character of colonies, relative arrangement of adjacent cells, and flagella position.

13. Anthrax organisms growing on an agar slant (Fig. 60). This whole whitish mass represents millions of cells which have developed in the time indicated from a few organisms placed upon the surface—so few that no organisms were visible even several hours after they were placed there with a transfer needle (Fig. 81). Such multiplication explains the formation of the whitish

FIG. 60.—Anthrax organisms growing on an agar slant, two days old. (After Moore.)

patches which develop on the throat in diphtheria, or the white "matter" which forms in a pimple or tiny abscess, for such patches or masses are bacteria plus mucus and dead cells of the tissue being attacked.

14. This microscope shows anthrax bacteria growing in the characteristic long chains commonly obtained in broth cultures

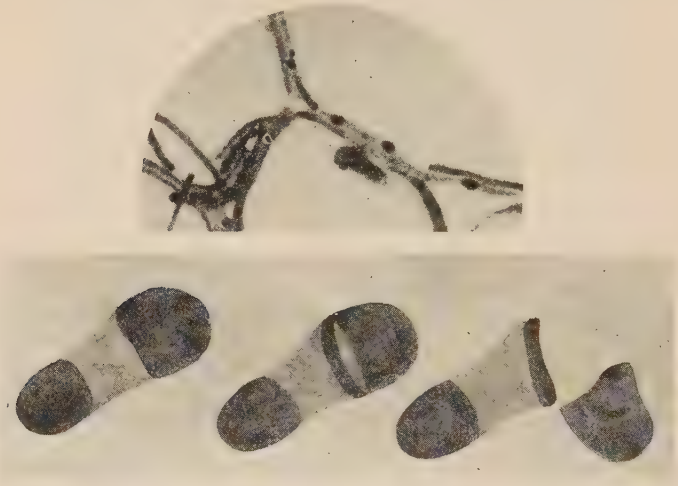

FIG. 61.—Nucleated bacteria. Above a nucleated bacillus, found in decaying teeth (Howe: Jour. of Med. Research). Below are shown stages in cell division of diphtheria bacteria, the division occurring in or near a nuclear (?) granule. (After Park, Williams and Krumwiede: Pathogenic Micro-organisms, Lea and Febiger.)

(Fig. 30, II), note magnification, 1200 diameters (x 1200); this means that each cell here seen must be divided lengthwise into 1200 equal parts to give you the actual size of a single cell. It is most difficult to picture a cell of such minute size and conceive of it as containing the normal cell parts (nucleus, cytoplasm) and performing such functions of living cells as the formation of enzymes, the splitting, and oxidation and storage of food substances; but every effort should be made to keep constantly in mind the physiological rather than the morphological characteristics of these minute cells. See p. 32, and p. 79.

15. For contrast with the rod-shaped cells already seen this

microscope (Fig. 62) shows some coccus organisms (magnification x 1200) lying in characteristic groupings: 2, 4, 8, etc. What is the most common group arrangement in the field shown?

16. An agar slant of a common yellow coccus organism, *Sarcina,* in which the organisms are characteristically in cubical

FIG. 62.—*Sarcina,* showing the compact packets of 8 or more, commonly found in this group or genus. (Morrey: Fundamentals of Bacteriology, Lea and Febiger.)

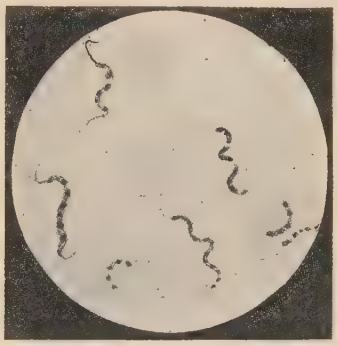

FIG. 63.—Large spirals magnified but 400 times or diameters. Note the variation in shape, due to the number and degree of the curves. Give one reason why these are probably protozoa rather than bacteria. (After Williams.)

packets of eight or more. Was the coccus organism seen in Exercise 15 above a member of this group or genus?

17. Large spiral organisms obtained from water from a shallow marshy pool (Fig. 63). Focus on the spots in these

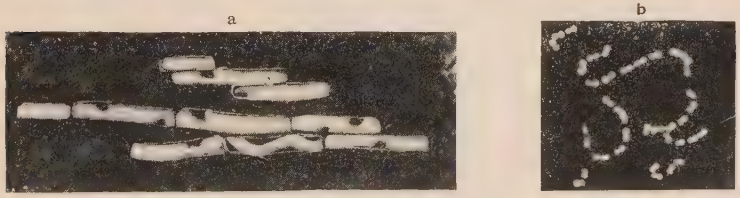

FIG. 64.—At the left, a common spoilage organism, *Bacillus subtilis,* lying unstained on an India ink background; on the right, chains of *Streptococci,* more or less twisted. In the latter, note the range in the size of the individual cocci; such enlarged (involution?) forms are not uncommon in old cultures. (See Fig. 48.)

large spiral cells; are they denser and darker than the cytoplasm, indicating that they are probably nuclear material, or are they refractile as is characteristically true of spores (and sometimes of vacuoles)?

18. The organisms seen in the previous exhibits were stained with various aniline dyes (methylene blue, gentian violet). A better idea of bacteria is probably obtained from such slides as that shown in Fig. 64, made by placing a loopful of the broth or agar culture in a drop of India ink stain. When mixed together the colorless or whitish bacteria may then be seen against

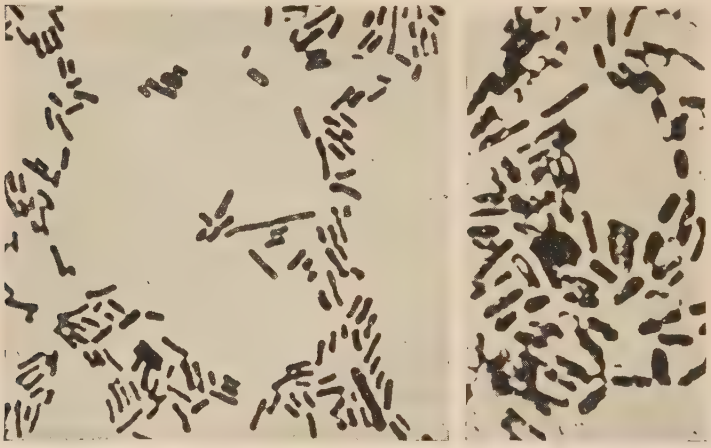

FIG. 65.—Variations in individual size and in arrangement (single, in pairs) in broth cultures. Left, typhoid organisms, right a spore-forming bacterium, *Bacillus megatherium*, often found in soil.

a dark India ink background. This first slide shows bacilli in the vegetative stage, no spores being present.

19. Several twisted chains of *Streptococcus,* also unstained, on an India ink background (Fig. 64).

20. Usually every small drop of a bacterial culture shows organisms of various ages differing, more or less, in relative size and proportions. Look over the field shown here (Fig. 65) until you have determined (1) the greatest difference in cell length shown, and (2) the absence or presence of spores in the oldest cells, and (3) indications of beginning cell division (indentation at margin) in cells not yet definitely divided into two.

21. Streptococcus organisms stained with a special stain to show the hazy capsule (Fig. 104, II) surrounding each organism.

These capsules are more or less confluent, and form a hazy margin along the whole chain.

22. Anthrax organisms from animal tissues (Fig. 40, II) and from ordinary laboratory media (Fig. 30, II) showing differences in the prominence of the surrounding capsules.

23. A drawing (Fig. 66) showing various types of bacterial cells based upon the number and arrangement of the flagella. To which type does No. 24 following belong?

Fig. 66.—Diagrams showing various types of flagella attachments. Flagella are not so helpful in naming or identifying organisms as might be expected because, besides being very delicate (easily broken off) and retractile (withdrawn under unfavorable conditions), flagella apparently may vary decidedly in both number and attachment in a given species.

24. Rod-like organisms, *Proteus vulgaris*, showing numerous long flagella (similar to Fig. 121) extending from all surfaces of the organism. Leaf through a text-book of bacteriology and find an illustration of a pathogenic organism having a different type or arrangement of flagella, and draw the type selected on the margin of this page.

25. Two types of spore production (Figs. 30 and 61, II). In which is the organism swollen at one end? In which is it usually swollen in the central zone? Look during the course for organisms which show no change in the cell outline with spore production.

26. The adjectives in the following list are commonly used in describing individual colonies as we find them growing upon such solid media as agar and gelatin.

pigmented	moist	spreading
mycelioid	cretaceous	effuse
mixed	opaque	granular
wrinkled	transparent	warty
concentric	radiate	small
thick-margined	solid	minute
umbonate	fimbriate	punctiform

On this table are a number of Petri dishes containing bacteria, yeasts, etc., from air or other sources, as indicated on the labels. On each plate one or more colonies have been ringed with a wax pencil. Use a hand lens, examining the colonies from the under side and then from the upper side to determine the surface and margin characters. Check on the list above the terms that describe these selected colonies. During the succeeding class periods be on the lookout for illustrations of the terms not yet obtained, and call the attention of the instructor to any new illustrations you are able to secure. (See following chapter for other *growth* characters.)

FIG. 67.—(a, b) Two photographs showing halves of agar plates, with a range in size and shape of the colonies often found in cultures of a given organism. How does the number of colonies in a given area affect the average size? Why are surface colonies often much larger than deeply imbedded colonies? Why are deep colonies often more or less compressed (whetstone shape)? (Left after Giltner: Microbiology; right, Johnson, Jour. of Agr. Research.)

27. Classification. Look through Bergey's Manual of Determinative Bacteriology and list the morphological characters used in describing bacteria. What activities or growth characters (pigment production, dissolving gelatin, etc.), are used? (See also Fig. 147.)

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(See also general reference list in Appendix)

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REVIEW AND STUDY QUESTIONS

1. Give four differences between bacteria and protozoa.

2. How do bacteria differ from such single celled plants as bakers' yeasts? What one character would positively separate bacteria from yeasts?

3. What morphological characteristics are used in classifying bacteria (see Fig. 147).

4. Illustrate what is meant by a "complex life cycle" among protozoa.

5. What different uses or functions do flagella serve in the life of micro-organisms?

6. What harmful organisms form spores?

7. What phases of bacteria and protozoa are engaging the attention of investigators today? (See Current Economic Bacteriology.)

CHAPTER V

BACTERIAL CULTIVATION AND ACTIVITIES

LECTURE AND READING OUTLINE

A. Metabolic processes resembling those of all other living cells

(A) Breaking down of complex food substances for use in cells
  a. Accomplished by simple cleavage, oxidation, etc.
  b. Attended by liberation of heat and energy

(B) Assimilation of materials into cell substance (cytoplasm, nucleoplasm)

(C) Synthesis of complex food and storage material (glycogen)

(D) Accompanying manifestations, often more strongly emphasized and so appearing more important than fundamental vital functions, such as (B) and (C) above. Such manifestations may be classified according to

  a Mode of formation
    (a) From the food materials quite directly; incidental to the breaking down of large molecules (proteins) preparatory to cell absorption (ptomaines)
    (b) Within the cell during metabolism and excreted in surrounding material (toxins, organic acids, carbon dioxide)

  b. Substances themselves
    (a) Chemical groups: acids, alkalies, amino-acids
    (b) Visible changes: gas accumulation, liquefaction (gelatin, cheese curd)
    (c) Characteristic odors or flavors as in wines, foods (butter: rank, fishy) or soil (freshly plowed earth: "smell of spring")
    (d) Poisonous character: toxins; these differ from ptomaines because
      1. Formed in bacterial cell
      2. Chemical structure not yet known
      3. Always toxic

B. Bacterial activities, generally listed as special vital phenomena

(A) Motility

  a. Due to flagella or cilia rather than to flexuous body, as in protozoa

  b. Microscope magnifies movement rate; although bacteria are magnified proportionately, our feeling of rate is affected by the apparent distance covered

c. Independent movement (direction, rate) and therefore different from Brownian movement, the vibrating, pivoting motion of tiny particles which lacks progression (see p. 113)

(B) Light

a. Sufficient to cause luminous appearance of culture and to take own photograph (Fig. 68)

b. Shared by crustacea and protozoa (ocean water) and molds (decaying roots and stumps, causing marsh lights, will-o'-the-wisp lights)

c. Characteristic of living substance

(a) Not in filtrate of luminous water or broth

(b) Salts present are important; luminous manifestations commoner in ocean, marshes, etc.; may occur in decaying salted fish or meat

(c) Not a constant character

Fig. 68.—Bacteria may give off enough light to affect photographic paper and so take their own pictures by merely placing photographic paper near them. Cultures of such organisms are luminous in the dark; phosphorescence in ocean water is due to bacteria and other light-emitting organisms. (After Lafar.)

(C) Heat

a. A common phenomenon; heat evolved by all living cells

b. May be shown in cultures by use of delicate thermometers

c. Partly responsible (compare share of molds, oxidation changes in green cells) for increased temperature in silo, piles of curing tobacco, coffee, etc.

d. Commonest illustration: steaming manure piles, where the heat due to bacteria causes the water to evaporate more rapidly than from the surrounding wet soil

(D) Pigment

a. Range of colors wide: purple, green, red, yellow
b. Lipochromes (chiefly); common in other plants (carrot) and animals (egg yolk)
c. Distinguished by difference in solubility: water, alcohol, etc.
d. No known importance (except bacterio-purpurin, which functions as chlorophyll does); recent studies of colored algæ show light absorption by pigmented area. See spirogyra in Fig. 69

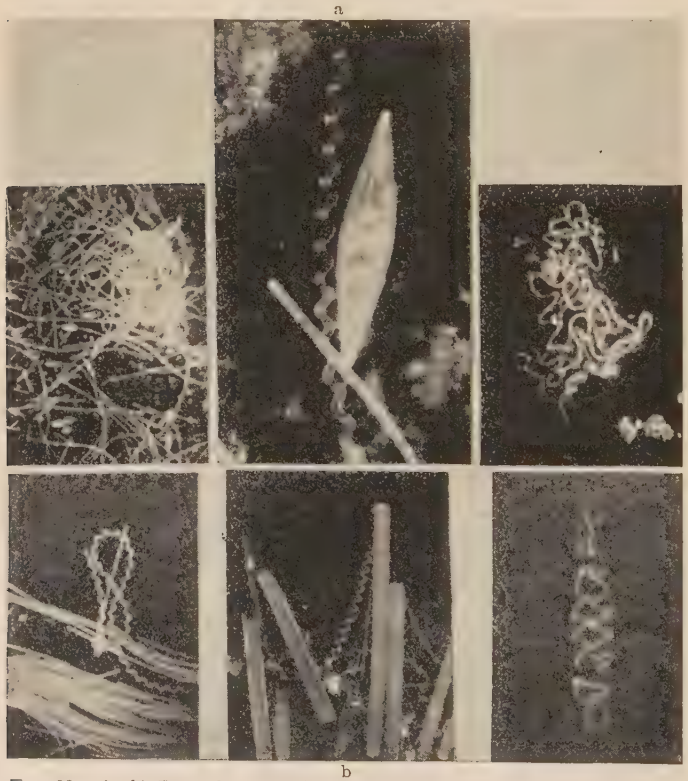


FIG. 69.—(a, b) Recently it has been demonstrated that chlorophyll and other plant pigments not only absorb light (see photosynthesis, Chap. I), but emit light after the original light is withdrawn. The above are actual photographs of algæ or simple water plants photographed (dark field) by Lloyd, 1923. Note the luminous twisted plastids in Spirogyra (see FIG. 31) in the lower right photograph. The mass of small globular cells is *Nostoc;* the spirals are types of *Arthrospira;* the straight threads are *Oscillatoria* (two species differing in size) and *Cylindrospermum.*

e. Color affected by oxygen (due to oxidation of excreted wastes; surface tension)

f. Presence erratic: affected by temperature, oxygen, food, antiseptics, etc.

DEMONSTRATION AND LABORATORY WORK

I MEASUREMENTS AND TOOLS

1. Temperature. The accompanying thermometer (Fig. 70) gives centigrade and Fahrenheit equivalents for the temperatures most often used or discussed in bacteriological work. In the middle column the main temperature groups are indicated: boiling, freezing, etc. At the bottom of the page are rules for converting one type to the other; these should be memorized for use when double scale thermometers are not available.

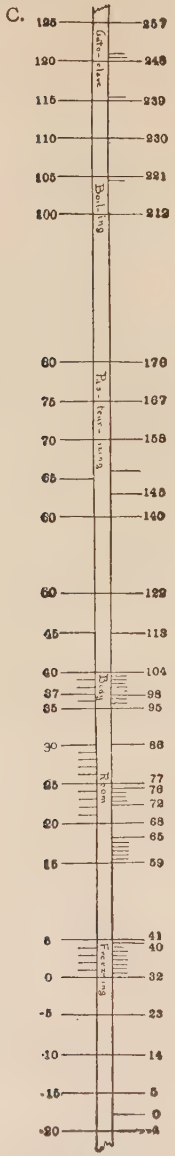
2. Length. The inch and its most-used divisions (1/8, 1/12) are too long for such tiny micro-organisms as bacteria, as it is, of course, necessary to have a unit of measurement small enough to be repeated in the length of the organism which is to be measured. The centimeter (1/100 of a meter) is subdivided into ten divisions: millimeters or thousandths of a meter. The millimeter is again subdivided into 1000 parts; each of these final divisions is called a micron (μ) or a micro-millimeter; a micron is therefore 1/10,000 of a centimeter or 1/25,000 of an inch.

1 in. = 2.54 cm. (See Fig 71)
or (reversing)
1 cm. = about 1/2.5 of an inch
1 mm. = 1/10 of one cm. = about 1/25 of one inch
1 μ = 1/1000 of 1 mm. = about 1/25,000 of one inch.

3. Weight. In bacteriological work the common unit of weight is the metric system unit, the gram (gm.); 1000 grams = 1 kilo (kilogram). Keep in mind that about 30 (28.8) grams make one ounce. How many grams in a pound? How does a kilo compare with a pound? How many ounces in a kilo? The gram itself is sometimes divided into one thousand smaller units or milligrams.

4. Volume. The liquid metric units are the cubic centimeter (c.c.) and the liter; 1000 c.c. = 1 liter. Since 1 cubic centimeter of water at 4° C. (or 39° F.) weighs 1 gram, we commonly use grams and cubic centimeters of water as interchangeable. How many grams in 1 liter of water? How would the number of grams in a liter of broth, agar, or carbolic solution, compare with this? To help visualize these volume measures, in Exercise 8 following, measure out several of the units or amounts often cited: 1 c.c., 2 c.c., 5 c.c., and 10 c.c. It will also be helpful to remember that a cubic centimeter is about 16 drops; a liter is a little more than a quart (a quart is about 960 c.c.).

5. Per cent. solutions. In making up ordinary solutions it is the common laboratory practice to use grams and cubic centimeters, somewhat interchangeably, especially for water. For example, in making up



To convert degrees Fahrenheit into degrees centigrade:

{ subtract 32, multiply by 5 divide by 9

To convert degrees centigrade into degrees Fahrenheit:

{ multiply by 9, divide by 5, add 32

FIG. 70.—Find centigrade and Fahrenheit equivalents for three different temperatures bacteriologically important.

FIG. 71.—Parallel scales comparing inches and cubic centimeters (See also Exercise 2, page 92).

a 5 per cent. sugar solution we weigh out 5 grams of sugar and add that to 95 c.c. of water. In handling such liquid disinfectants as carbolic acid, the custom is to take 5 c.c., by bulk, of the heavy acid liquid, and add 95 c.c. of water; here we have, of course, more than the desired 5 per cent. solution; the difference, however, in making up disinfectants, is on the safe side, making the solution stronger, not weaker. For more delicate processes (making up stains) the directions usually state specifically for each substance (liquids, powders, crystals) whether it is to be measured by weight or volume.

6. Securing solutions of designated proportions.

(a) It is often necessary to change a solution of a given strength to another strength or per cent. Often disinfectants, etc., bear such labels as, "To one quart of water add one-fourth of a bottle," etc., or "To any bulk of disinfectant add four times that amount of water." There will be many occasions, however (*e.g.*, making stains), when you will need to work more exactly, in definite percentages. The "rule of three" or proportion method may always be relied upon, not only to give the required per cent., but in the desired bulk. For example, if you need 500 c.c. of a 5 per cent. solution of formalin, and your stock formalin is a 40 per cent. solution, your mathematical arrangement would read: 40 per cent.: 5 per cent.:: 500 c.c.: (?) or 62.5 c.c. You would then take 62.5 c.c. of the 40 per cent. solution, and add water to bring it up to 500 c.c., thus securing 500 c.c. of a 5 per cent. solution.

Sometimes the terms of the proportion are carelessly put down in the wrong sequence, yielding ridiculous results, and beginners are advised to check up their answers with what seems sensible. For example, in the above problem we wish to secure a 5 per cent. solution which is ⅛ of the strength of the 40 per cent. stock solution. The amount used therefore, the 62.5 c.c., is ⅛ of the total bulk, 500 c.c.

b. In making solutions of the required strength, it is usually much quicker to follow a very old but little-used laboratory rule: To dilute to any strength take a number of units (oz., c.c., etc.) to correspond with the dilution desired and add water (or designated solvent) to bring the bulk up to the number of units indicated by the original strength. To illustrate: to get 12 per cent. alcohol from 95 per cent., pour 12 c.c. of alcohol into a graduate, and add enough water to fill your graduate up to 95 c.c. You will then have 95 c.c. of 12 per cent. alcohol. How can you make 2½ per cent. formalin from 40 per cent.? This method is not so well adapted to expensive materials. Why not?

7. Needles. Nothing is as satisfactory as platinum wire for bacteriological needles (Fig. 81). Size 28 is a very good weight of thickness, because it is heavy enough to give a little resistance or spring when it comes in contact with the slide, and yet thin enough to cool off quickly after flame sterilization.

Another great advantage of platinum is that its expansion on being heated is so slight that it does not crack the glass holders, as do

most of the types of wire available for this purpose. The best substitute for platinum among the inexpensive wires is a chromel A wire, size 28, which has proved very useful for laboratory work, and which causes less breakage of the glass holders than any other substitutes.

The making and repairing of glass-handled needles is avoided by a new screw holder (Fig. 81), which is split to allow insertion of the

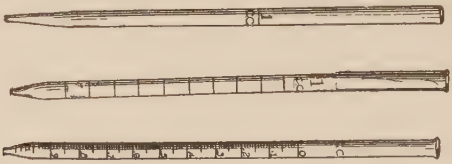


FIG. 72.—Three cubic centimeter pipettes. The upper one is "volumetric graded" only, but the lower ones are graduated for measuring parts (tenths, hundredths) of a cubic centimeter.

wire. A special device allows sufficient movement of a burr or nut to fasten or liberate the wire, but prevents the nut from working off the handle.

8. Pipettes

(a) This exhibit (Fig. 72) includes several different types of pipettes, used in making dilutions of different strengths for milk and water plates. Three different kinds of pipettes holding one cubic centimeter are shown. One cubic centimeter fills the space from the pointed tip to the upper level indicated by a faint scratch around the tube and the mark, 1 c.c. (These are made to deliver 1 c.c.; it is not necessary to blow through the pipette unless the tip is clogged. This is much more likely to occur in pipettes used for bacteriological materials (broth, dilutions, blood) than for ordinary chemical substances.)

In the second pipette this space is subdivided into tenths of a c.c.; in the third, the ultimate divisions are hundredths of a c.c.

(b) Practice drawing one c.c. of water into a pipette; let it out by tenths, stopping completely at each tenth mark. (See caution following.) If the pipettes provided do not have such subdivisions, mark several levels with a wax pencil and use those marks as guides. Repeat, until you are sure you can control the amount leaving the pipette, so that at the next lesson the sterilized pipettes may not be wasted by avoidable errors.

Caution. Use the first finger, not the thumb, to cover the mouth end of the pipette. Using the thumb tightens the hand too much for the delicate manipulation often necessary in bacteriological work. Hold the pipette near the top, so that the first finger rests comfortably on the top of the pipette. In regulating the outgo, lift the knuckle part (not the nail end) of the finger first, as the skin near the nail is harder and gives a less delicate control than the part near the middle of the joint.

(c) This is a new type of pipette (Fig. 73) which protects the user. The two bulbs take care of excess liquid (cultures, etc.), and do not allow it to enter the mouth. A cotton plug is sometimes used at the mouth end of the ordinary pipette to slow the rise of the liquid and so protect the worker. Some find that device unsatisfactory, and it is often quite troublesome to insert the cotton and to clean it out after using. The double bulb pipettes are, of course, more expensive.

(d) Special pipettes such as these (Fig. 73) are often used for making milk plates. The narrow bore makes it possible to measure very small amounts, such as 1/100 of a c.c., with considerable accuracy, because even that small amount fills a considerable length of the tube—from the tip up to the cross line shown in the illustration. The larger the apparent bulk or unit used, the more accurately the eye can measure off these small amounts.

FIG. 73.—The upper pipette is the special pipette used in making "direct counts" of milk. The space between the figures near the tip and the tip holds 1/100 c.c.
The lower pipette has two bulbs to catch any excess drawn into the tube, thus protecting the worker and also preventing mouth contamination of the material.

II MEDIA (SEE APPENDIX, ALSO)

1. Meat Extract Broth. The medium most commonly used in general laboratory work is a broth made of meat extract to which are added peptone and common table salt. The usual proportions are:

Beef-extract (such as Liebig's)	3 grams
Peptone, pure (Witte's or Difco)	10 grams
Sodium chloride (common salt)	5 grams
Water (tap water)	1 liter

The solid ingredients are added to the liter of water, and the mixture is heated for a few minutes, just enough to dissolve the substances (about 60° centigrade). This does not boil away the water and so change the proportions advised above.

For such elementary bacteriological work as is given in this text, broth made according to the above directions may be used without any special attention to its acidity or alkalinity. Acid and alkali relationships are, however, of supreme importance in real bacteriological work, and a brief discussion of this problem is given in the Appendix.

In filtering, the filter paper may be folded in any of the common ways used by pharmacists or other workers. It is customary to moisten the filter paper after it has been placed in the funnel. This favors the passage of all the substances in the broth mixture.

After filtration, the material is placed in tubes or flasks, properly plugged and sterilized. This may be done by heating it

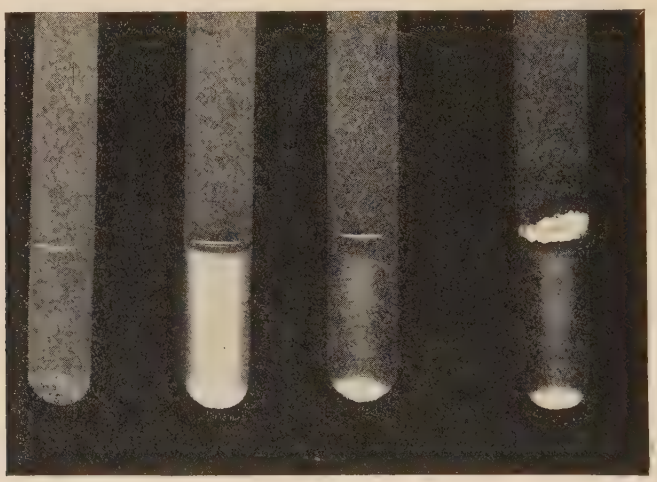


FIG. 74.—Types of growth in broth: left, control tube of sterile broth; second, very cloudy or turbid; third, slightly cloudy with sediment; and fourth, with a pellicle as well as sediment. (Morrey: Fundamentals of Bacteriology, Lea and Febiger.)

once in the autoclave, keeping it at 15 pounds pressure or 125° centigrade for 15 minutes. A streaming steam sterilizer such as the Arnold sterilizer may be used instead; in that case the material should be heated (at 100° C.) on each of three consecutive days for 20 or 30 minutes.

If the material is to be sterilized in the autoclave, it is not necessary to sterilize the tubes before filling. If, however, the discontinuous method of sterilization is used, the test tubes should be plugged and sterilized before they are filled, as well as after the medium has been put into them.

2. Solid Media. To nutrient broth, such as is described above, a solidifying substance may be added, thus enabling us to study

the bacteria in small separate masses (colonies), such as those shown in Figure 100. The solidifying substances commonly used are gelatin and agar.

(a) Common table gelatin may be used, but a purified form, which is clearer or less opaque and which contains fewer unknown organic substances, is preferred. This gelatin is added to the broth in proportions of 10 to 15 per cent.; in warm weather the higher percentages are advisable, as gelatin liquefies at rather a low temperature, making it impossible to determine whether the liquefaction is due to the room temperature or to the enzymes of the bacteria under observation. Bacterial effects are usually described with regard to the results obtained (1) in test tubes, (Fig. 74), or (2) on Petri dishes, where the shape of the individual colony is more readily observed.

Gelatin should be heated as little as possible, as overheating interferes with its solidifying. Add it to the hot broth, and filter as soon as it is dissolved. Tube in sterile test tubes and heat in the Arnold for 30 minutes on each of three successive days. Cool immediately (icebox or running water) on removing from the Arnold to solidify the gelatin; then leave at room temperature until the next heating.

Two different types of liquefaction are shown in Figure 75. Later, during this course, inoculate a tube with your special culture (p. 111) to see if it will afford a third type.

(b) Agar. This is a glutinous substance which is obtained from such seaweeds as the red varieties observed along our own coasts. When seaweed is placed in fresh water a stringy or slimy exudate may be observed. More or less purified seaweed extracts are used as solidifying substances in bacterial media, very small amounts being sufficient, 1 to 1½ per cent. Such extracts are dried and sold in various forms: in shreds or stringy masses, in coarse granules, or as fine powder.

Some of the types of agar on the market do not dissolve readily on heating. This means that they must be boiled for some time, which is tedious and is attended by the danger of burning or boiling over. Such long boiling also evaporates much of the water and changes the concentration of the various food materials, as mentioned earlier. It is much simpler and easier to dissolve beforehand an appropriate amount of agar in a minimum amount of water (15 grams of agar to 200 c.c. of water is a good proportion), and add such dissolved agar to the other ingredients.*

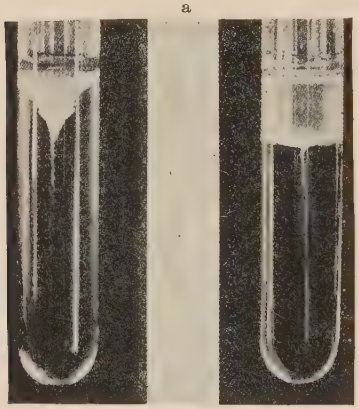


FIG. 75 a.—Two different types of liquefaction; left, funnel-shaped (infundibuliform); right, in horizontal layers (stratiform). (Reduced from Sackett, Colorado Agricultural College.)

FIG. 75 b.—A little water usually collects at the bottom of freshly slanted agar. Flooding the slant surface with this water before inoculation often gives a more favorable substratum for bacterial growth. (Ricker.)

Bring the broth to a boil and add to it 1½ per cent. agar, melted as described above. Filter the mixture while hot, through a hot, wet filter made of absorbent cotton, which may be kept warm and moist in the Arnold until ready for use; the funnel

*This makes an extra amount of water—200 c.c. to a liter. But if the broth is brought to a boil and kept boiling until the agar is thoroughly mixed and dissolved in the broth, experience has shown that we lose an amount of water about equivalent to the extra water added to the agar. If the final bulk is less than the recipe, tap water may be added. Such agar stock re-dissolves more readily than before, and may be melted in a few minutes in the autoclave or in 20 to 30 minutes in the Arnold, while the other materials are being brought together.

may be left in the Arnold during filtration to hasten the filtering process.

In a cold or dry filter the cotton detains much of the agar, and does not give the proper concentration in the filtrate, yielding a medium which lacks sufficient agar for making Petri dishes of the required consistency. The agar provided by the best manufacturing houses has little, if any, effect upon the acidity, and it is not customary to titrate or correct the medium (acidity) after adding the agar (see Appendix).

When agar is kept in test tubes a little liquid often collects at the bottom of the slant (Fig. 75). This is "expressed water" and is not due to a liquefying enzyme.

Agar solidifies at about 38° centigrade, and remains solid until it is brought to practically boiling temperature. Agar is therefore a much more convenient substance for ordinary bacterial work than gelatin, but it lacks the differential value of gelatin, as practically none of the common bacteria liquefy it.

(c) Other Types of Food Materials or Media.

1. Sugar Broths. Bacteria (*e.g.,* the colon bacterium) are often grown in media containing sugar, usually 1 per cent. of such sugars as dextrose or lactose. Utilization of such sugars by bacteria is indicated by the formation of acid or gas or both. (This and the following exercises furnish examples of bacterial enzymes; see Exercise 21, p. 43.)

To test for acid we may use litmus or phenolphthalein. These tests are made in various ways. Litmus, for example, may be added to the food substance when it is made up; and as the organisms develop, their activity is indicated by changes in the color of the litmus. Blue litmus agar, for example, turns red as *coli* organisms develop in it; and bluish litmus milk turns pink as streptococcus multiplies in it.

Often the formation of acid is determined by applying the indicator to the medium after growth has occurred: by touching the tube contents with a piece of blue litmus paper, or by titrating to determine the exact amount of acid formed (See Appendix).

To test for gas, fermentation tubes, such as those used in the yeast experiments, are generally used. The gas collected is tested for

carbon dioxide and any residue is usually tested for hydrogen (Fig. 76). See also p. 66.

Gas production may be demonstrated, also, by deep inoculations of organisms into tubes of sugar agar. Gas production is indicated by bubbles of gas in the tube (Fig. 77); this method, of course, does not lend itself to determining the kinds and proportions of gas formed.

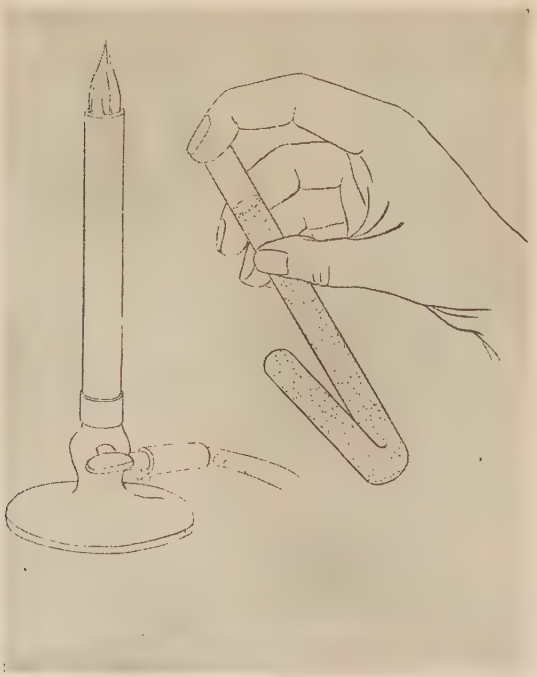


FIG. 76.—Tilting the tube of *coli* to bring the gas to the open end to test for H will enable us to complete the H—CO_2 ratio. If two-thirds of the gas is CO_2 and the remaining third proves to be H, the $\frac{H}{CO^2}$ ratio will be ½.

Gas formation may be demonstrated in ordinary straight test tubes of liquids. After the broth or milk has been inoculated, melted paraffin is run into the tube. This hardens

into a plug on the surface of the medium. If the organism being studied can grow without air, this method may be used to show gas

FIG. 77 a.—Stab inoculations are made to show ability of a given organism to grow without oxygen (first tube on the left), or to demonstrate the gas-forming power of an organism, such as *coli* (second tube). (Morrey: Fundamentals of Bacteriology, Lea and Febiger.)

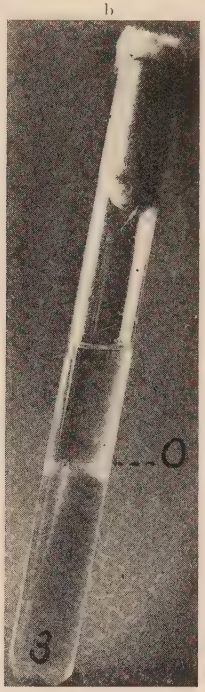


FIG. 77 b.—A culture of *coli*, a facultative anaërobe, growing deep in the agar, in spite of the oil covering on the surface. (Ricker.)

formation, for the plug is pushed upward as gas forms (Fig. 78).

Milk.

(a) Litmus is often added to milk or broth to determine whether or not the organisms form acid or alkali from a given sugar, such as milk sugar.

Compare these litmus-milk cultures of the colon bacterium and the hay bacillus with the sterile tube of litmus milk. Which organism formed acid? Which alkali?

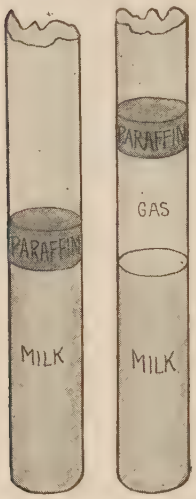


Fig. 78.—Objectionable gas-forming anaërobic spore-bearing organisms which survive pasteurization may be detected by incubating paraffin-covered samples of the milk; note the change (right tube) in the position of the paraffin layer when gas is formed.

(b) There are various changes in milk which may be observed just as well in tubes of plain sterilized milk, uncolored by litmus. Some organisms produce a curd, which may form either an apparently homogeneous mass or may separate (Fig. 79), expressing a liquid (whey). This solidifying effect may be followed by a digestion of the milk (peptonization), or such digestion may occur without preliminary solidification. What changes have been made in the milk by the cultures exhibited here (the colon organism, the hay bacillus, etc.) ?

3. Potato. Strips of potato are sometimes used to show whether or not bacteria use such starchy substances or break down the cellulose composing the cell walls. The potato strips are washed thoroughly in running water to

remove all loose starch grains and thus leave the strips with clean sharp edges. Water is added to the level of the lower edge of the slant surface of the potato strip (Fig. 80), and the tube is sterilized in the autoclave.

(Sterilization in the Arnold is too slow; the longer period which is necessary softens the potato so that its mushy condition makes it difficult to determine whether or not the bacteria disintegrate the potato substance.)

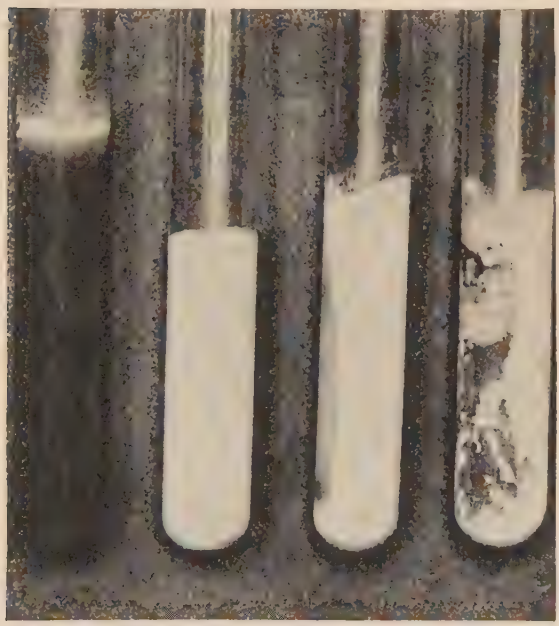


FIG. 79.—Four types of changes produced in milk are shown here: (1) digestion (*subtilis*); (2) solid curd (*coli*); (3) curd with whey expressed (old *coli*); (4) tight fibrous curd with much whey expressed (unidentified bitter organism from milk). (Ricker.)

In many cases pigment formation is more easily determined on the white background afforded by the potato than on agar.

4. Blood. Blood is often added to media such as agar to provide the necessary food sub-

stances. Some pathogenic bacteria cannot be cultivated satisfactorily on laboratory media unless blood or serum is added.

These special substances may be used also to differentiate between the organisms. Some streptococci, for example, make a greenish pigment on such blood agar plates; other streptococci dissolve or destroy the red blood cells, so that the opaque red plates of blood agar become clear-spotted due to the transparent area surrounding each bacterial colony (Fig. 2, II). This ability is usually closely related to the pathological effect of such bacteria when growing in the human or animal body, the destruction of the red blood cells being very harmful.

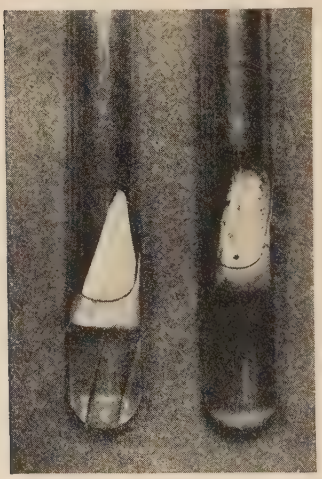


Fig. 80.—Potato cut into sharp-edged pieces and sterilized with water filling the lower part of the tube. A piece of glass may be used to raise the potato in the tube and thus allow comparison of total growth and related disintegration of potato (accumulations at base). Note in the right-hand tube (*megatherium*) the less transparent character of the water, the accumulated sediment, as well as the less sharp edges of the potato. (Ricker.)

Occasionally effects somewhat similar to this dissolving action on blood are noticed on agar: the translucent agar becomes clear in the surrounding area, as in the accompanying plate (Fig. 121).

5. Oxygen Relations

(a) Most bacteria need oxygen for their development. The few types that grow

best with little or no oxygen are grown by using a special apparatus, which allows us to exhaust the oxygen from

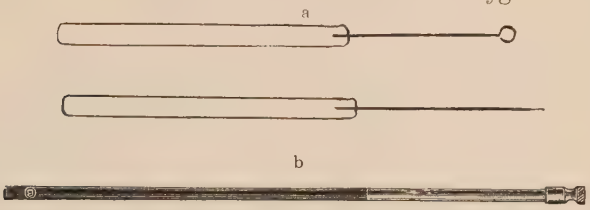


FIG. 81.—Two types of transfer needle handles: (a) glass rods heated to allow the insertion of a platinum wire, and (b) a handle of a composition material with an adjustable patented device for holding the wire in place. (See Exercise 7, page 95.)

the air or to substitute instead of air another gas, such as hydrogen (Fig. 82).

(b) The ordinary classroom materials, however, may be used to indicate differ-

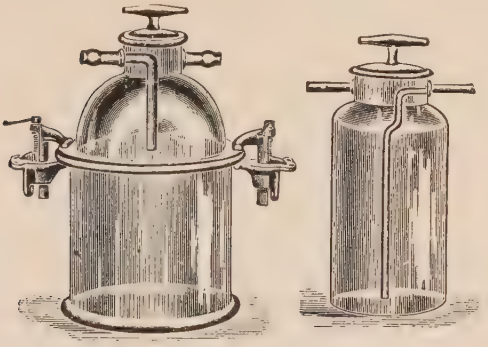


FIG. 82.—Drawings showing two types of anaerobic culture jars, each with a pair of tubular openings or attachments which enable us to replace the original air (oxygen) content by any desired medium, *e.g.*, hydrogen, after the cultures have been sealed in the jar. For single agar tube cultures pyrogallic acid may be used to absorb the oxygen, a sufficient amount being placed in the bottom of a bottle and the tube inverted into the pyrogallic acid.

ences with regard to the need for oxygen. Tubes of agar may be inoculated with the straight needle, which places the organisms deep down in the sub-

stance. Some organisms will fail to develop with the small amount of oxygen available in the depths of the agar (Fig. 77).

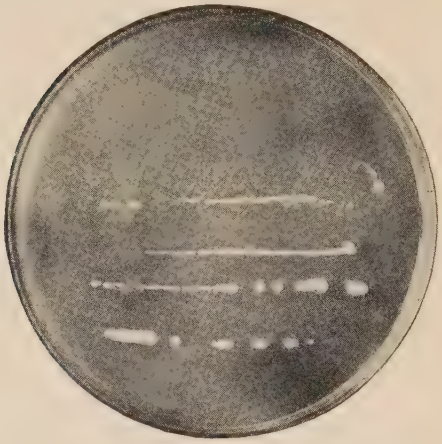


FIG. 83.—A streak plate. Various arrangements are used: a single long continuous spiral, several parallel streaks (as here shown), latticed or cross-barred lines, or streaks radiating from the initial contact of the needle (or swab) with the agar (Schlagenhauf). Pour plates are shown in Figures 67 (right) and 85.

The path of the needle allows entrance of a small amount of air, of course. This may be lessened by inoculating the agar when it is in a slightly softened condition —almost completely solidified; it will then fill up the track of the needle as it hardens. The access of outside air is thus prevented, and the bacteria are left in an anaërobic pocket in the middle of the tube.

(c) A more satisfactory method of sealing the organisms from the air is adding some inert oil * such as liquid vaseline to the top of the medium (Fig. 77).

(d) Differences in growth (amount, pig-

*Oil is, however, sometimes described as having an opposite effect on oxygen absorption and transfer.

ment formation) due to oxygen relations may be shown by inoculating a Petri dish with such a pigment-producing bacterium as the prodigiosus organ-

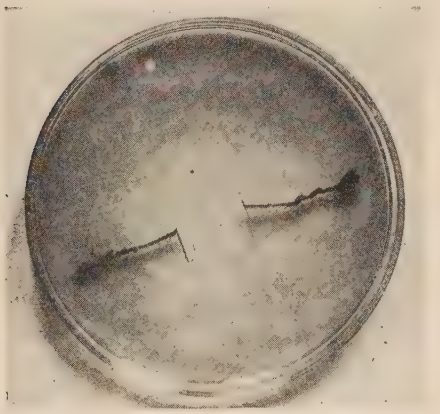


FIG. 84.—The red "prodigiosus" organisms (*Serratia*) were streaked across the middle of this plate, and a cover-glass was then pressed firmly over the center of the streak. Note the poorer growth where the cover-glass limited the oxygen supply. (Ricker.)

ism (Fig. 84). The exhibit shows a streak of this organism growing on an agar plate. Immediately after making the streak, a cover-glass was pressed down firmly against the agar, across the middle of the streak. Note how much heavier the growth is at the ends of the streak; note also that the characteristic red pigment shows only where oxygen could reach the organisms.

III CULTURES AND THEIR MICROSCOPIC EXAMINATION

a. Isolation of pure cultures.

(a) "Fish" a distinct or well-separated colony from an agar plate (*e.g.*, one of

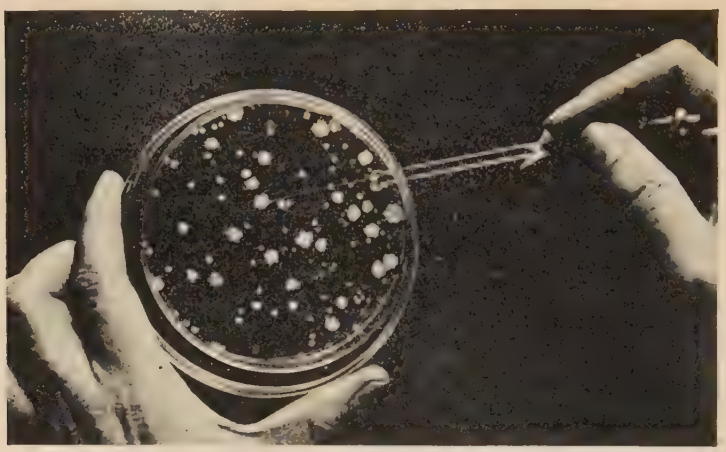


FIG. 85.—In "fishing" we work under the upper half of the Petri dish, as shown, to prevent air organisms from falling into the lower half of the dish. (Green and Ricker.)

FIG. 86.—Note the plug held at right angles between the last two fingers. It is taken out by the hand that holds the needle so as to cause as little movement as possible in the air, at the open end of the tube. Why? (Holden and Ricker.)

the air-exposed plates, p. 155) to broth, as shown by the instructor (Fig. 85).

To help insure the purity of the culture, flame the needle thoroughly, hold the tube horizontally (not vertically) when unplugged, and flame the neck of the tube on opening and again on replugging. (This last flaming makes it less necessary to flame the surface of the cotton plug, unless it actually touches an object or surface while out of the tube, for the replaced plug comes in contact with the hot glass.)

(b) Incubate at room temperature until the next class session. (If no apparent growth (gross appearance), incubate again at 37° C.)

(c) Examine a smear at the following class session, to determine the purity of your culture; (dry, fix, and stain as described on p. 115). Further evidence of purity may be obtained by plating out the culture. This may be done (as demonstrated by instructor) by streaking a loopful of broth on a sterile agar plate (streak plate method); or by adding a small amount of the culture to a tube of melted agar (cooled to about 45° C., to prevent killing the organisms), mixing well, and pouring into a sterile Petri dish (pour plate method). Use one of these plating methods (Fig. 83) with your "pure culture" at the next class period, and compare your results with those obtained by the rest of the class to find the advantages of each method of plating.

b. Running a series of pure cultures.
To demonstrate the acquisition of a reasonable amount of technique, the student is asked to isolate a pure culture, to carry it through

several successive transfers in broth, and to demonstrate its purity at each stage, by microscopic examinations and by occasionally streaking or plating it out, as additional indication of its purity.

Rods vary so with age that coccus organisms will be more satisfactory for beginners. The colored colonies on your air plates are quite likely to be cocci or yeasts, either of which may be used. Carry this series on as rapidly as your class work permits; twenty-four hour intervals are sufficient, but as long as a week may elapse between transfers. It will be wise to keep one or two back transfers, for a contamination may unexpectedly be found to run back one or two cultures. In this case, it will be necessary to go back to the last pure culture, or to re-isolate by streaking out the impure culture (if relatively few of the undesired organisms seem to be present).

c. Demonstrating the wide distribution of bacteria.

Inoculate a tube of broth with material from one of the various places discussed in class: doorknobs, paper money, coins, pencils, finger nails, skirt-braids, gloves, skin, etc. A convenient method is to invert a tube of broth, thus wetting the inside of the cotton plug. The moist plug may then be rubbed over the skin, knob, or other object selected, replaced in the tube, and washed off by the broth.

Incubate for two days, one day at room temperature and one day at 37° C., to be sure to provide a favorable temperature for the organisms thus obtained.

Which source failed to yield organisms? What type of organisms (coccus, rod) seems most indicative of recent human contact? What percentage of the class cultures shows spores? Do these results indicate any desirable change in any personal habits? Any definite menace to health?

d. Examination of sputum etc.

In such examinations, one usually has in mind a definite organism, *e.g.,* diphtheria in suspected sore throats, typhoid in fever patients, or tuberculosis with clinical symptoms of that disease. Streptococci are found in a majority of normal human throats, and we may therefore look for that organism, instead of a distinctly pathogenic type. The presence of given organisms in such material may be determined in several ways, which vary greatly in the time interval demanded:

1. By direct examination—rubbing a throat swab (as shown by the instructor) over a glass slide, which is then fixed and stained in the usual way (p. 115).
2. By streaking the throat swab over an agar plate, as shown. The plate is then incubated, and colonies which resemble streptococci are examined by taking a little material from a given colony on the tip of the needle and mixing it on a slide in a loopful of sterile water or broth; this "smear" is then fixed, stained, etc. (p. 115). If preferred, the colonies thought to be streptococci may be fished to broth and incubated before examination.
3. By inoculating the collected material into a special or selective medium which favors the growth of the organism looked for, such as blood for pathogenic streptococci. (Other selective media in common use are brilliant green agar for typhoid, neutral red, lactose broth (p. 166) for the colon bacterium.)

e. Motility

(a) Brownian movement. Mount a little carmine in a drop of water. Note under the microscope the dancing motion of each particle—motion without progression (see p. 90). This phenomenon is often noticed in suspensions of small particles, and is named (for an early investigator) Brownian movement.

This movement without any change in relative position of these small bodies is attributed to (1) the vibrating motion characteristic of molecules in general, and so exhibited more or less by any

tiny particles; or (2) to differences in surface tension, a torsion or twisting resulting from the unequal wetting of the irregular surfaces of the particles.

(b) Motility. Place a small drop of the bacterial culture provided, on a cover-glass, and invert over the cavity in the hollowed-out slide supplied for that purpose (Fig. 77, II). Seal the edges with vaseline, as shown by the instructor, to prevent the organisms from shifting their position as the liquid evaporates. Focus carefully with the high power. (A wax pencil mark on the lower side of the cover-glass will help locate the proper focal distance.) Watch (high power) until you are sure it does not sway too much (too large a drop) nor shift (imperfectly sealed). Find (1) organisms that show only Brownian movements, and (2) other organisms that are truly motile, and change their relative positions.

f. Staining bacteria

(a) Mixing stains. Most of the bacterial stains are aniline dyes, and are usually purchased in quantities in a powdered condition. These powders are ordinarily made up into stock solutions, commonly saturated solutions in 95 per cent. alcohol; tilting the stock bottle will allow us to determine whether or not we have a saturated solution, considerable undissolved material in the bottom of the bottle being taken as evidence of saturation of the liquid.

These stock solutions are then made up into weaker watery solutions, when they are usually ready for use. For specific directions for a given stain, see the Appendix.

(b) Mordants. Certain chemicals increase the penetrating power of stains. Such substances are called mordants. Carbolic acid is often used as a mordant in connection with fuchsin. These mordants may be added after the stain is mixed and diluted, or the stain may be dissolved directly in the mordant solution; for example, one direction for making fuchsin stain reads: 3 parts saturated alcoholic solution of fuchsin to 20 parts of 5 per cent. carbolic acid.

Another common mordant is potassium hydroxide, which is used in making up methylene blue stains.

Heat has similar mordant action, and such stain-resistant bacteria as the waxy-coated tuberculosis organisms are stained by applying heat (water bath, flame).

(c) Staining exercises

1. Methylene Blue

(1) On a clean slide place a loop of broth containing bacteria. If the bacteria are in a solid medium, place a drop of sterile water (or broth) on the slide, and with a sterile needle transfer a minute portion of the culture to the water, spreading it throughout the drop.

(2) Allow the film to dry at room temperature.

(3) Fix the organisms to the glass by passing it three times through a flame. Too great heat will shrivel them. (Why?)

(4) Add enough stain (methylene blue) to cover the film well, and let it stand 1 to 3 minutes.

(5) Wash in water; with clean slides the film will show the stain, and the rest of the glass will be perfectly clear.

(6) Dry, by draining or by pressing gently, film side down, on a piece of filter paper.

(7) Add a drop of water, cover with a clean cover-glass and examine.

2. Fixing agents other than heat.

(1) Heat is not always a satisfactory agent for fixing micro-organisms to the slide, *e.g.*, milk, for which we employ alcohol. (See p. 64.)

(2) Some stains, *e.g.*, Jenner's, are used in strong alcoholic solutions, and do not require any preparatory "fixing." Most of these require care to prevent the deposition of sediment on the slide, (filtering the stain, covering to prevent evaporation of the alcohol during staining, etc.).

(3) A "fixing" agent in quite common use is corrosive sublimate (saturated water solution).

To fix organisms by this method, dry the smear at room temperature in the usual way, and cover it with corrosive sublimate for one (or two) minutes. Usually the smear will whiten somewhat, as white of egg does when coagulating by heat. Wash promptly (to avoid destruction of cellular character) and thoroughly (to avoid crystal formation when the dye is added.) Dry again at room temperature, and stain as usual with methylene blue. Compare this with a control slide fixed by heat. Which is better suited to the organism you are working with?

3. India ink as a background for unstained bacteria. (See Appendix)

Place a loop of India ink on a clean slide. To this add a loop

of the bacterial culture, mixing it thoroughly, so that there is a range of thick and thin places on the slide, some of which will be dark enough to make a favorable background, yet thin enough to allow sufficient light to pass through to illumine the organisms. When dry, add oil and examine. Do not add water; that dissolves the ink. What are the advantages of this stain with regard to time and the type of laboratory equipment necessary? Would it be helpful in emergencies or in poorly equipped laboratories?

This stain does not remain sterile, and is not therefore well adapted to common class use, because it is difficult to tell whether the organisms seen in such a slide are represented in the material being examined or whether they are due to contamination of the stain by a previous worker. (The common aniline dyes, because of their very affinity for the bacterial cell substance, interfere with cell growth and kill off bacteria, should careless handling by a previous worker contaminate the solution.)

4. Carbol-fuchsin. After you have grown accustomed to the methylene blue stain, compare that with the carbol-fuchsin, by making two smears of a given culture and staining each with a different stain. Fix both smears with heat as usual, then put a drop or two of sterile water on the smear to be stained with fuchsin; this lessens the precipitate so often

seen in connection with this stain. Add the carbol-fuchsin stain, wash it off in one-half to one minute, and drain dry as usual. This is a very much more avid stain than methylene blue, and has a greater tendency to over-stain the material; there are also often formed

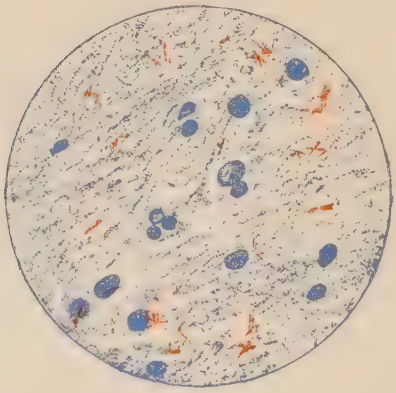


FIG. 87.—Human tuberculosis organisms, still retaining the red fuchsin stain, despite being bleached by acid or alcohol. Only bleached cells (other bacteria; white corpuscles) take the second or counter stain (blue in this case).

on the slide many small globules of stain, which may confuse the beginner.

5. Decolorizing stains with acid and alcohol. Organisms differ in their affinity for stains and the ease with which they relinquish stains with which they have combined. Alcohol and acids (nitric, hydrochloric) are commonly used as decolorizing or bleaching agents in this connection (Figs. 87 and 88).

(1) This smear of tubercular sputum was stained with warm carbol-fuchsin (See Appendix) to allow the stain to penetrate the waxy coating of the tuberculosis organisms. The slide was next bleached (with 1 per cent. sulphuric acid in water, or with 2 per cent. nitric acid in alcohol), and then washed and dried as usual. Note that the organisms have retained their color (Fig. 87). Such organisms are called acid-fast.

Any other organisms in the saliva which are not acid-fast will lose the stain entirely, and be colorless. To show them up, a counter stain is used—a weaker, watery stain of some contrasting color, *e.g.*, methylene blue. In such a double stained slide, therefore, the tuberculosis organisms are red, and the other saliva organisms are blue.

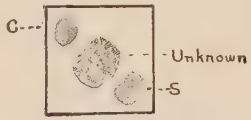


FIG. 88.—Organisms which are known to be either Gram-positive, S (*subtilis*), or Gram-negative, C (*coli*), are used in testing the staining characteristics of an unknown organism. If the known organisms come out positive or negative, as expected, the results with the unknown are accepted.

(2) Gentian violet (see Appendix) is another differential stain now in common use; with it, Gram used alcohol as a decolorizer. Organisms that keep the violet stain in spite of the decolorizing power of alcohol are termed Gram-positive; those that lose it, Gram-negative. Is your pure culture organism Gram-positive? Find in one of the larger texts on bacteriology a list of Gram-negative and Gram-positive organisms. Of what value is such a list?

Name one common disease organism that is Gram-positive? Gram-negative?

IV. NAMING UNKNOWN ORGANISMS *

An unknown organism is studied under the microscope to determine its shape, motility, size, and other characteristics, such as growing in chains, in pairs, etc.; and. when a pure culture of an unknown organism is assured, it is then inoculated into various food materials for observing its growth, and the changes or effects it produces.

Such descriptive charts as those issued by the Society of American Bacteriologists (Fig. 89) contain names of the various food materials in which organisms are usually cultivated for determining their characteristics, and also lists of the various types of results obtained, including colony appearances on gelatin and agar, and the turbidity, sediment formation, and other effects in broth.

The unknown organism shown in the accompanying tube has been stained and placed under the microscope exhibited next. Determine for yourself the shape of the organism and the absence or presence of spores.

In the next microscope you will find a hanging drop of a fresh (24-hour) culture of this organism, mounted as described earlier (Fig. 77, II) to allow you to determine whether or not it is motile.

Note the changes or results in the various media produced by this organism, giving such facts as amount of growth, pigment, character of sediment in broth, acid production, gas formation, etc., for the various tubes and plates of plain broth, common milk, gelatin, agar, sugar broths, etc.

The temperature of incubation was 37° C. in all cases except where room temperature is definitely stated.

Use these results in a key for classifying bacteria (see Appendix) and find the genus to which this organism belongs. Next, in Bergey in that genus, trace the organism down to the species name, thus obtaining for the unknown organism a double name, such as *Bacillus subtilis*.

(See the reference list for the preceding chapter as well as the general reference list in the Appendix)

REVIEW AND STUDY QUESTIONS

1. What vital processes are common to bacteria and all other living cells?

2. What special vital activities are prominent in phosphorescent molds and bacteria? In souring milk? In ripening Camembert cheese? In blue milk?

3. Read over the directions for making broth, sugar broth, etc. What essential nutritive substance or substances is supplied by each ingredient?

4. How may we determine whether or not oxygen is essential to the growth of an organism?

5. How may we determine the kinds of bacteria present in spoiled foods? In pus? In normal fermentation (sour krout, silage)?

* For this the instructor may set up a demonstration composed of microscope slides and cultures of any desired organism, as described. The Appendix shows how the information obtained from such a series of exhibits may be used to determine the name of an unknown organism.

6. How may we show the ability of organisms to use such special test substances as starch? Lactose? Peptone?

7. What are we really testing for when we test bacterial cultures for gas, hydrogen, acid, etc.?

8. What are mordants? Why are they used in bacteriology?

9. How do decolorizers aid in bacterial identification?

10. How would you proceed to demonstrate the purity of a given culture?

11. What are the uses of streak plates? Of pour plates?

12. What physiological phases of bacteria and protozoa have recently been shown to be important in sanitary or medical tests? (See Current Economic Bacteriology.)

CHAPTER VI

PHYSICAL AND CHEMICAL AGENTS AND CONDITIONS

(Mainly from the viewpoint of disinfection and sterilization)

No sharp distinction is here drawn between physical and chemical agents. How impossible that would be can be seen by recalling the discussion of plasmolysis: the withdrawal of water from a cell is itself a physical change—but when it brings about an unfavorable concentration of cell substances (salts or organic acids), the effects produced upon the cell are chemical.

A. General discussion of effects produced upon micro-organisms

(A) Effects on cells vary with intensity of concentration of agent (heat, electricity, acids), producing

a. Death of the organisms
b. Inhibition of their growth
c. Attenuation of their powers
d. Changes in their characters (type of motility, kind of pigment, etc.)

(B) Agents classed to indicate action or effect on cells

a. Substances causing complete cell destruction are termed germicides and bactericides. (The latter indicates destruction of bacteria only, while a germicide must kill protozoa as well; since most bacterial spores are very resistant, a bactericide is practically always a germicide)

b. Disinfectant. This term implies the killing of all infectious organisms. A disinfectant is not necessarily as strong as either of the two preceding, because most bacteria causing infection or disease do not form spores. A disinfectant, therefore, for typhoid or diphtheria or other home or hospital use, is not necessarily a germicide or a bactericide. Similarly, "sterilize" and "disinfect" are not synonyms

c. Antiseptic, more limited than either of the above terms. Distinguish between antiseptic and asep-

tic substances or conditions. (A bandage sterilized by heat may be called an aseptic bandage. A wound free from infectious organisms or evidences of decay (sepsis) may be described as in an aseptic condition. A bandage containing corrosive sublimate or other drug which can inhibit bacterial growth is called an antiseptic bandage)

d. Deodorizer, a substance which contains or liberates something which absorbs, replaces, or destroys objectionable odors; it has no definite standing as regards its disinfecting or germicidal value. Deodorizers as such are therefore of little value in hygienic procedures; in fact their use tends to cover up rather than remove irritating or objectionable conditions

(C) Effects on cells; due to interference with normal cell processes by

a. Plasmolysis; loss of the water necessary in cell metabolism

b. Concentration of cell substances; the withdrawal of water by plasmolysis may cause such a concentration of ordinary cell substances (oxalic acid, etc.) as to irritate or injure the cell beyond recovery

c. Coagulation or similar chemical changes affecting the vital plastic condition of the cell. Compare effect of heat, alcohol or corrosive sublimate on cell protoplasm, white of egg; coagulation indicates extreme stage in loss of plastic condition

d. Oxidation changes, such as are obtained by ozone Unfavorable oxygen conditions include

(a) Oxidation changes in food supply, rendering it less soluble or usable

(b) Oxidation of the cells themselves

e. Toxic and irritating effects on cell substances, not included in the above changes

B. Principal agents used

(A) Electricity

a. Difficult to separate from its accompanying effects, which may include

(a) Increase in temperature

(b) Electrolytic products: acids, ozone, chlorine, which are themselves destructive agents

b. Practical applications few or limited: has been used in the treatment of wine for elimination of undesirable organisms without complete destruction of favorable forms. The bactericidal effects obtained in water are due to oxidation through the ozone formed. The electrical sterilization of milk, though possible, has not been found practical; neither has the disinfection of sewage (passage of sewage between two iron plates charged with a strong current, which must pass through the sewage flowing between them)

(B) Gravity

a. Indirect effects (oxygen supply, effect of sunlight) through change in position or depth in liquid. Such effects are lessened by power of many bacteria to change position by flagella action. Direct effects not great, because of relatively light specific gravity of most bacteria. Gravity sedimentation of bacteria is relatively slow, being modified by
   (a) Currents in the liquid
   (b) Rate of water flow
   (c) The buoying effects of retained gas, as in the pellicle on broth cultures

(C) Agitation

a. Destructive effect lessened by small size of organisms and consequent ease of movement through molecules of suspended liquid

b. Disintegration may follow protracted violent shaking; agitation sometimes employed in making bacterial vaccines

(D) Pressure: contradictory statements regarding pressure due to the fact that one author may be dealing with compression of liquids, and another with the compression of gases applied after compression to liquids containing bacteria

a. Compression of liquids
   (a) Effects slight because
      1. Most liquids have little compressibility

2. Most liquids contain relatively small amounts of gases which would be thus forced into solution to interfere with bacterial growth; oxygen (air) practically only active gas in ordinary liquids

(b) The effects obtained depend upon the amount of compression and the consequent increased concentration of gases that can affect contained bacteria

(c) Illustrations of slight effects due to compression of liquids themselves

1. Decay goes on at great ocean depths, showing that saprophytic organisms can exist under great pressure applied to liquids
2. Pressure designedly applied to liquids containing organisms may have little effect upon the contained bacteria
   (1) Pressure of three thousand atmospheres applied to liquids for four hours did not kill the majority of molds and bacteria tested
   (2) Some organisms survive even when subjected to two thousand atmospheres for 3 days
   (3) Non-spore-bearing bacteria are killed in 14 hours by 6000 atmospheres; spore-bearing organisms by 12,000 atmospheres

b. Compression of gases

(a) Effects depend upon type of gas, varying with

1. Affinity of gas for cell substances and therefore its power to interfere with normal cell activities
2. Ability of a given gas to pass into solution in water, and so enter the bacterial cells

(b) Main practical applications found in such gases as chlorine or oxygen which may be compressed and then liberated in the water, killing most of the contained organisms. (See treatment of water by ozone and chlorine.) Less active sub-

stances such as carbon dioxide may be used; charged water is often found to be sterile; 52 atmospheres pressure of carbon dioxide will sterilize water in 24 hours; charged milk, it is claimed, will keep several days longer than the same grade (raw, pasteurized) of uncharged milk. (Milk inoculated with typhoid, dysentery and diphtheria was subjected to CO_2 gas, 150 pounds pressure; in 24 hours 98 per cent. of the organisms were destroyed, and complete sterility was obtained in 4 weeks.)

(E) Light

a. Sunlight

(a) Effects proportional to directness and intensity of light

1. Seasonal variations, illustrated by such comparisons as destruction rates for the prodigiosus bacterium: by 1½ hours exposure, summer sunlight, and 2½ hours, winter sunlight
2. Intervening matter lessens penetration

(1) Air: clouds and dust
(2) Water: turbidity due to suspended soil particles or organic matter
(3) Mucus or other covering of bacteria: as in sputum and feces

(b) Spores sensitive to light effects: the oily contents seem to favor the chemical action of light

(c) Inhibiting and germicidal effect greatest toward the chemical or blue end of the spectrum: ultra-violet light with strongest chemical effect, now yielding good results in treatment of deep-seated infections (joint and gland tuberculosis, bone infections). Solariums are established at high altitudes where the proportion of ultra-violet rays in light is greater. Treatment (tuberculosis farms, etc.) aided by stimulation of body tissues (skin, affecting respiration, circulation)

b. Artificial light
   (a) Ultra-violet light
      1. Quartz globes necessary: glass interferes with passage of ultra-violet rays; new "fused quartz" has great promise as glass substitute with sunlight as well as artificial light
      2. Practical applications demonstrated
         (1) Treatment of water: drinking water; swimming pools
         (2) Bacterial preparations (vaccines)
   (b) Radium and X-rays
      1. Plant experiments (radium) indicate stimulating effect upon growth with many of the green plants tried
      2. Treatment of pathological tissues* (cancer, tuberculosis) brings up necessity of considering effects in relation to
         (1) Stimulation of normal body tissues
         (2) Disintegrating effect on such tissues
         (3) Destruction of disease-producing cells

(F) Removal of water, or drying

a. Plasmolysis effects, as described earlier
b. Effects related to rate. The accommodation of the cell to drying depends upon the rate of water removal, quick drying bringing about a very rapid destruction of the cell; slow drying (old agar slants or when placed in specially prepared chambers) may allow retention of vital powers for months, even by organisms not possessing spore-forming power
c. Action commonly combined with sunlight effects, as in destruction of bacteria in air

* The cures effected today are the results of long experience, and cases should be taken only to best specialists in this work. This does not belittle the wonderful results obtained with such light treatment, but emphasizes the necessity of securing experienced workers.

(G) Food: proportion and kind, may be viewed as a combination of various physical and chemical agents. Under this may be considered such minor topics as

a. Amount of food: survival periods in ordinary water—even distilled water—sometimes explained by amount of food material carried over on surface of the bacteria
b. Concentration of food elements, as well as irritating substances retained in cell, is affected somewhat by the amount of water present in the surrounding food material, and determines the consequent normal or plasmolized condition of the cells
c. Acidity and alkalinity. Slight variations most important in many organisms. Leprosy forms an example of preference for alkaline medium (−1.5 to phenolphthalein. (See Appendix)
d. Lack of essential food elements
    (a) Vitamine requirements. Recent investigations indicate dependence of many organisms upon food containing vitamines; difficulty in continued cultivation under laboratory conditions of certain organisms may be explained on this basis
    (b) Dependence upon certain minerals, such as phosphorus, potassium, calcium, indicates same necessity for balance of inhibiting and stimulating factors as with ordinary cells (green plants, human tissues)
e. Many bacteria found to be extremely sensitive to substances necessary to or utilized by other bacteria; *e.g.*, some soil bacteria are inhibited by the presence of ammonia, while others need it for their growth
f. The bacterial by-products or wastes accumulating in surrounding food material may affect life of cell
    (a) Growth may cease even while unused food is left; organisms die as acids, etc., accumulate in media
    (b) Broth cultures usually die more quickly than agar cultures, partly due to difference in contact with wastes

(H) Temperature

The growth and survival limits of some organisms show a wide temperature range, (*e.g.*, 6° to 60° C. for *Bacillus subtilis*), while other organisms are very sensitive to slight variations. The maximum growth temperatures may reach as high as 80° C. (hot springs); this resistance to the coagulating effect of heat has been explained by presupposing anti-coagulating enzymes

The main part of the following discussion of temperature is limited to the germicidal effects of unfavorable or destructive temperatures

a. High temperatures
   (a) Moist temperatures more effective than dry
      1. Penetrating power greater
         (1) Air and air spaces in materials, such as bedding
         (2) Cells themselves
      2. Coagulation point of proteins lower with moist heat. Compare different coagulating temperatures of egg albumen: when very diluted it coagulates at 56° C., when only 18 per cent. water at 80°-90° C., and when 10 to 6 per cent. water at 125° to 145° C. The low water content of spores is related to high temperatures necessary to destroy them
   (b) Methods of securing high temperatures
      1. Without moisture
         (1) Hot air oven for sterilizing glassware, etc.: temperature of 180° C. for one hour or more sometimes insufficient; compare with uniformly effective results with moist heat (autoclave) of 125° C. for 15 minutes
         (2) Flame for platinum needles or burning infected clothing, diseased plants, etc.
      2. With moisture
         (1) Pasteurizing, as in milk. Pathogenic organisms in vegetative stage killed by 30 minutes at 62°-65° C. Film on surface of milk affects temperature, the film temperature being lower than that of the milk below the film; avoid by covering milk during

pasteurization or by stirring in film

(2) Boiling—effective for vegetative stage or organisms lacking spores, since coagulation of such pathogenic bacteria occurs at 80° C. or below. Really unnecessary to *boil* water, but such directions may be given instead of "heat to 80° C.," as then no thermometer is needed

(3) Streaming steam (100° C.), as in Arnold sterilizer (Fig. 90). Dishes or other infected articles may be subjected to streaming steam (in washboiler, covered pail) instead of being actually immersed in water (boiling). A half-hour period is usually sufficient to insure penetration of the steam in ordinary conditions requiring disinfection.

To disinfect material containing spores or to sterilize bacterial media (agar, broth) the discontinuous or fractional method of sterilization may be used: streaming steam applied for half hour periods on *two or three different days,* thus killing the vegetative stages and catching any spores originally present, as they change to the vegetative stage

—Simple apparatus; the top of a washboiler is usually loose enough to allow some movement or streaming of the steam; a pail with a cover or another pail inverted over it may also

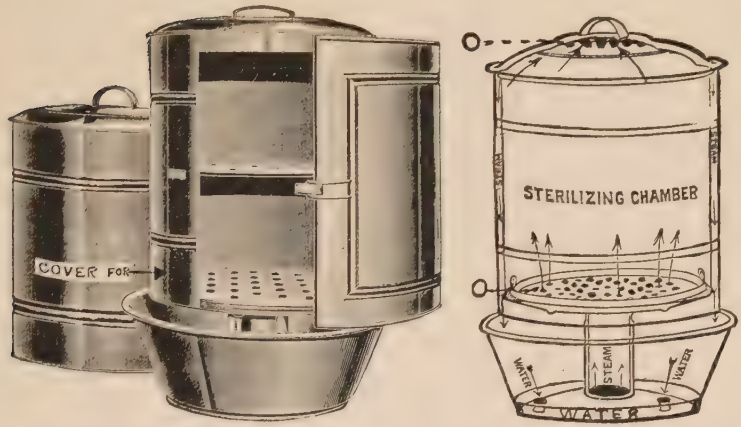


FIG. 90.—Two illustrations to show the construction of an Arnold sterilizer. In the right-hand drawing arrows indicate the upward course of the steam, which later passes down inside the outer cover, which is entirely separate in this type of Arnold. (See Fig. 91.)

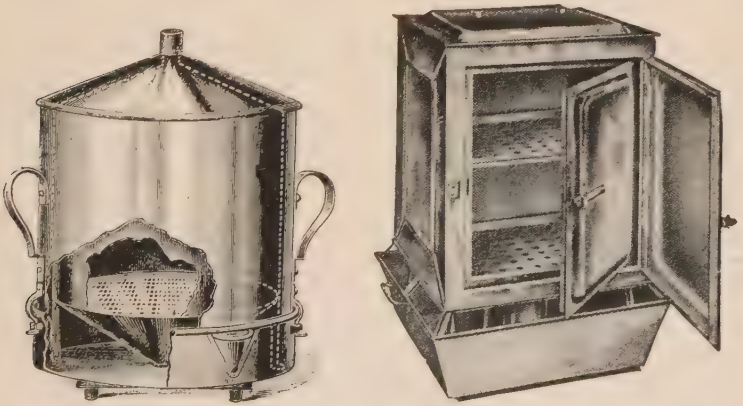


FIG. 91.—Two other types of Arnold sterilizers. In the one on the right the outer and inner jackets are not separable; steam passes up into the top and down at the sides just as shown in Figure 90.

be used in emergencies for killing undesirable organisms (on dishes, clothing, etc., from the sick room)
—In institutions, attachment of a boiler to the steam heat system is possible, giving a sterilizer without the risk of boiling dry occurring in the Arnold sterilizers, washboilers, etc.

(4) Temperatures above 100° C.

a. Without pressure; obtained through raising the boiling temperature by addition of salts, or by substitution of oil instead of water, as a surrounding medium.

Little value except addition of salt to water in cold pack method, canning corn. Oils not practical, as common oils are inflammable, costly, or else have objectionable odors. Cooking temperatures of meats or of fish cooked in fat (country method canning sausage or older commercial method of canning fish) are very much higher than 100° C.

b. Temperatures secured by heating under pressure; autoclave and pressure boiler hold steam under pressure, resulting in increased molecular action (and pressure) of the steam particles and also increased temperature as the pressure increases (15 pounds pressure = 125° C., etc.)

Steam under pressure has expansive power or power to do work and is described in various textbooks as live steam. The more the steam is heated, the greater the resulting molecular activity (radius of movement) of the steam molecules, and the fewer the steam molecules that can be accommodated in a given space. This causes the precipitation of some of the steam back to water into the water reservoir, leaving less water represented in the steam above the water. The more such steam is heated the less water is found represented in it. Such superheated steam is therefore called dry steam when contrasted with steam formed without pressure, (streaming steam) as in the teakettle, washboiler, or Arnold. While dry or superheated steam contains less water and is therefore from that point of view slightly less effective, the increased temperature obtained compensates for the difference in water content

b. Low temperatures
   (a) Freezing
      1. Freezing kills 75 per cent. ordinary bacteria in water
      2. Effect of ice splinters in surrounding watery medium on bacterial cell membranes a factor
      3. Expansion of watery substances within the cell precedes freezing (water expands as temperature falls from 39° F. to 32° F. or freezing); this expansion may rupture the cell membranes
      4. Thawing with consequent expansion as temperatures rise may also have destructive effect (rupture cell membranes); rate of thawing important, may demand too rapid cell adjustment

5. Increased concentration of salts, etc., in surrounding medium as water is withdrawn (changed to ice) may affect micro-organisms

(b) Temperatures below freezing of little practical importance; expensive to obtain and have surprisingly low germicidal effect. Investigations report (1) typhoid and diphtheria not impaired by 190° C. below zero and (2) 10 per cent. of organisms surviving 170° below zero. Lack of germicidal effect explained by relative lack of moisture present, intense cold being dry cold, and water being necessary for effect on cell substances, just as in high temperature effects, as described earlier

(I) Chemicals used in disinfection. The values of the respective chemicals used depend upon (1) affinity for organic matter, (2) stability of combination once made and (3) the ease with which the efficiency can be increased

a. Methods of increasing efficiency

(a) Increasing the concentration, or using more of the stock substances in making up the solutions: 5 per cent. carbolic advised for destroying spore-forming bacteria although 3 per cent. carbolic kills streptococci, staphylococci, etc., in one minute

(b) Raising the temperature; all chemical action is more rapid as temperature increases. Heating or boiling in weak dilution (soaps, formalin), etc., is as effective as higher concentrations used without heat

(c) Adding water

1. Penetration of substances into cell membranes depends upon the presence of water. Absolute alcohol, therefore, less effective than 95 per cent. alcohol
2. Volatile substances, such as alcohol, may be held longer in contact with desired surface or substance, thus favoring chemical combination; this is thought to explain the good effects obtained with low percentages (40–50 per cent.) of alcohol

3. Gases may have their penetrating power —through bacterial membranes as well as through intervening room or air space— increased by the presence of water. Sulphur or formalin room disinfection therefore much more effective when water vapor is present. (Boiling kettle in room, heating liquid formalin over flame)

(d) Combining two chemicals

1. Increasing the absorption rate or the chemical action of a given chemical by the presence of a second, *e.g.,* the addition of alcohol to water solutions of corrosive sublimate. The chemical action of two such substances may, besides, liberate a third effective factor: formalin and lime generate heat; and formalin and potassium permanganate liberate oxygen, as well as generate heat

b. Types of chemicals used as disinfectants

(a) Mineral acids. Of little practical use in home and hospital because of affinity for clothing or other fabrics and metals (containers); also injurious to body tissues

(b) Alkalies, such as ammonia and lime, active because of unstable condition; used chiefly as disinfectant for fecal material

(c) Metallic salts, such as bichloride of mercury or silver nitrate. These two have very destructive effects upon body tissues, which necessitates handling with extreme care and under medical advice only

(d) Coal tar disinfectants, carbolic acid or phenol, lysol, cresol, and other coal tar derivatives. Many of these (Hycol, Neko, CN) are called high-coefficient disinfectants because they are stronger or more highly efficient than carbolic acid, formerly considered the standard disinfectant

(e) Gases in common use

1. Oxygen as oxygen, or as ozone (see water disinfection)
2. Chlorine applied as a gas or a compound liberating chlorine gas (See water disinfection). Follow current trials with chlorine, in treating respiratory infections.

3. Formalin. Most widely used for room disinfection (See the following demonstration)

(f) Essential oils

1. Thymol, oil of cloves (See demonstration)
2. Turpentine. Too irritating for use except in emergencies

C. Practical phases of disinfection. Value of a disinfectant related to cost, ease with which put into available form (solutions, required dilutions), relative attraction for other albuminous substances, and risk attending handling, as well as actual disinfectant values

(A) Handling risks

a. Labels. Injurious effects upon hands, tissues (throat) fabrics, and metals should be definitely indicated by labels, etc.

b. Addition of harmless dyes or colors to distinguish presence: methylene blue to color corrosive sublimate tablets or wood alcohol

c. Distinctive shapes for poisonous substances or containers: square or coffin shapes suggested for corrosive sublimate tablets

d. Other methods of preventing mistakes: bell around bottles, label-bearing box lids not detachable, etc.

(B) Present attitude toward room disinfection

a. Concurrent rather than terminal disinfection of all infectious or infected materials (discharges, dishes, bedding, towels) should be emphasized

b. Terminal disinfection

(a) Of patient before return to society: bath, shampoo, mouth and nose treatment, fresh clothing, etc.

(b) Room disinfection

1. Thorough cleaning of room, utilizing sunlight, fresh air, and ordinary cleaning methods (soap and water, sunning of rugs, hangings, bedding). Fresh surfaces obtained by papering, oiling, or varnishing woodwork, etc., advised following tuberculosis
2. Chemical disinfection (formalin)

(1) Psychological value: to prevent undue alarm in diseases where transfer method not certain (infantile paralysis)

(2) Other values: to protect those who are to clean room after pulmonary tuberculosis (removing carpets, hangings, etc.); to destroy disease-carrying insects (lice, mosquitoes, fleas, etc.). Formalin still holds high place as bacterial disinfectant; steam alone often used for boats; hydrocyanic acid now much favored for vermin, if a tear gas is added to eliminate accidents

(C) Wound disinfection

a. Fresh and surface wounds—treated with iodine, alcohol, etc., to kill organisms entering wound at time of injury (see Demonstration list)

b. Infected wounds, which cannot be made "surgically clean," are sometimes flushed with a mild disinfectant, such as the Carrel-Dakin solution, liberating chlorine and inhibiting or killing bacteria without undue irritation of the surrounding tissue. This is continued as long as a microscopic examination of the wound shows heavy bacterial infection (more than 5 per field), or until the experienced eye finds the tissues healing normally

DEMONSTRATION AND LABORATORY WORK

1. Apparatus used in destroying bacteria

(a) Dry heat

The ordinary gas oven may be used as a substitute for the hot air sterilizer for sterilizing test tubes, Petri dishes, etc. The latter has better insulation—an asbestos covering and a good lining forming a complete air jacket.

For sterilizing glassware, as well as media, we use the autoclave. It costs less (for gas), does not char plugs, etc., and does not need to be watched so carefully, nor for so long a time. Hot air sterilizers should be held at 180° C. for at least an hour, while an autoclave is more uniformly effective if held at 125° C. for 15 minutes.

(b) Moist heat—not under pressure

1. Two views of Arnold sterilizers (Fig. 90) both of which use streaming steam, 100° C. The lower part has a closed chamber for water, causing it to heat more rapidly. The steam rises through the perforated base into the upper chamber. What finally becomes of it?

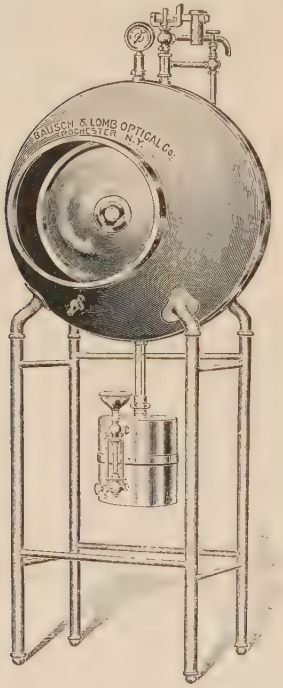


Fig. 92.—A self-locking door is shown in this autoclave; compare the clamp device for closing in Figure 93.

The more recent ones have this extra cylinder, a cover, which makes an air space, one to two inches wide, around the inner steam chamber. What effect does this cover have on the constancy of the temperature inside the sterilizer?

2. In some laboratories where the type of heating system makes it possible, streaming steam is secured by connecting the steam pipes with a copper boiler furnished with an outgoing pipe for condensation water, much as in the case of steam radiators. This is an excellent system, giving quick service most of the working year and costing much less in large laboratories than running individual Arnold sterilizers.

3. In our laboratory we use an improvised streaming-steam sterilizer (for sterilizing special media, killing old cultures, etc.). It is a tin ham boiler with a copper bottom. (Ham boilers have proportionally broader bottoms than washboilers.) Such boilers

give at least twice the sterilizing space at less than half the cost of an Arnold sterilizer; the cheaper (tin) ones do not rust out so fast as the double-bottom Arnolds, and they last eight to ten years in our laboratory. What does such a sterilizer lack to insure uniform, effective results?

(c) Moist heat—steam under pressure

1. Autoclave of an early type composed of a single cylinder or jacket (Fig. 92)

The early types of autoclaves had this circular door, shutting from the inside. The greater the inner pressure, the more firmly the door is pressed against the outer rim; it locks itself, therefore, autoclave. Steam may be generated from the water in the bottom of an autoclave, or steam may be piped into the autoclave from a separate but attached boiler, or from the heating system of the building itself.

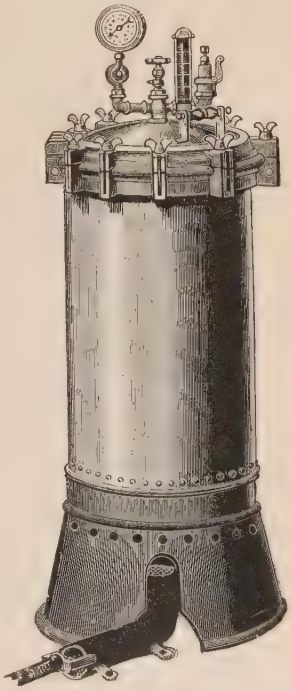


Fig. 93.—A single jacket type, two or more quarts of water being placed in this just as in the pressure cooker (Fig. 137).

2. One of our autoclaves is the single jacket type (Fig. 93). Where is the steam generated? How is the autoclave kept closed against the increased pressure of 15 pounds? After consulting the instructor, explain the action and value of the safety valve.

Autoclaves should not be opened until the

gauge drops to zero (ordinary atmospheric pressure). What may be the effect of opening it before that point is reached?

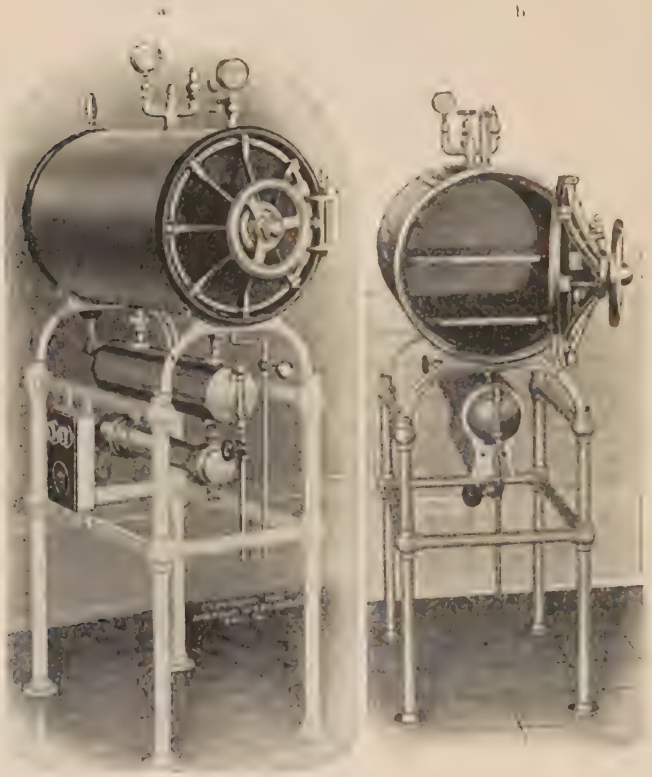


FIG. 94 a.—A popular type of autoclave which has a double wall or jacket is shown here on the left. The door is double, well insulated to help compensate for its lack of a steam chamber. Note the steam generator below. (American Sterilizer Company.)

b. The open autoclave shown here is very similar in appearance, but has only a single wall or jacket. Which type is better? (American Sterilizer Company.)

3. This autoclave (Fig. 94, a) has an outer jacket in which moist steam circulates, while the steam which later passes to the inner chamber is superheated and so rendered drier. Sometimes in

the single jacket type you will find much water of condensation on and in the sterilized glassware; it often leaves the cotton plugs very wet, also. This moisture deposit is less in the double

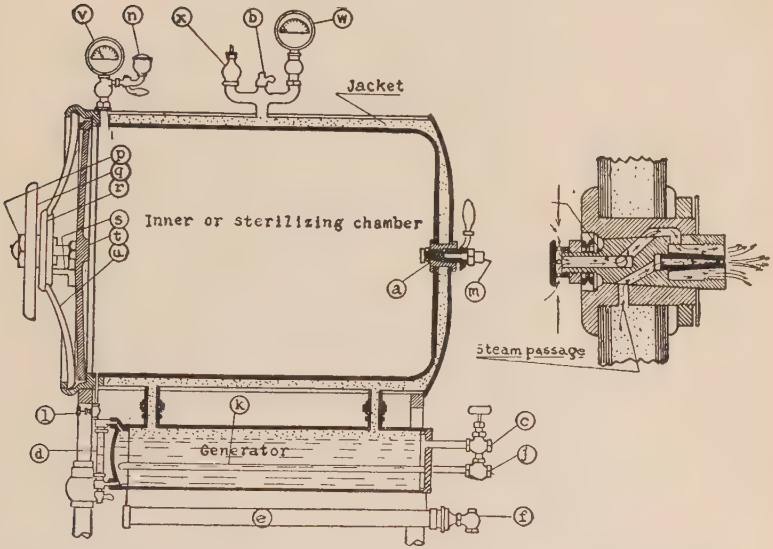


Fig. 95.—A diagram through a double jacket autoclave. The passage of steam through the special exhaust nozzle (m) is shown in the enlarged drawing to the right. The door and its parts are indicated by q to u; other parts by a, operating valve (3-way); b, air cock to jacket; c, water valve; d, gauge glass; e, gas burner; f, gas cock; j, steam valve; k, steam coil; l, air and drain valve on inner chamber; m, exhaust nozzle; v, pressure and vacuum gauge on inner chamber; w, pressure gauge on jacket; x, safety valve. (American Sterilizer Company.)

jacket autoclave. Note the steam generator attached below the autoclave.

4. An interesting application of the autoclave to household use is the pressure boiler (Fig. 137). This is practically like the single jacket autoclave. The shape of the base makes it possible to use this boiler with any form of stove (gas, wood, coal). Its chief value lies in the time reduction in the slow cooking processes usually accorded beans, meats, grains, etc. The small size usually sold for home use makes canning a very slow process. There are two advantages, however: (1) the shorter heating does not produce the "mushiness" of the discontinuous method of steril-

izing; and (2) the higher temperature kills the more resistant spores which survive in other methods.

(d) Drying, Pressure, Light and Freezing

1. Photograph illustrating the inhibiting or germicidal effect of sunlight (Fig. 96).

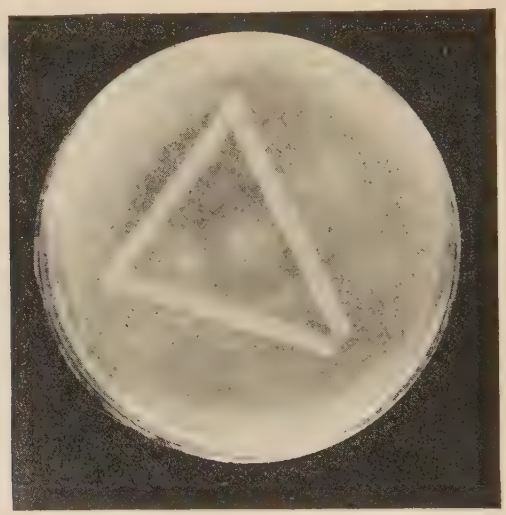


FIG. 96.—The germicidal effect of sunlight is shown by comparing the heavier growth in the triangle, due to the protection afforded by three strips of paper pasted on the plate, which was exposed for one hour to the sun, May 26, 1924. (Holden.)

2. Look through the recent pamphlets and books listed at the end of this chapter and find confirmatory, illustrative, or contradictory information regarding the material given under the four headings in (d) above.

(e) Chemicals

1. From the following section * of this chapter and your reading, select disinfectants for ten different, practical conditions, such as: (1) cut finger, (2) diphtheria dishes, (3) typhoid excreta, (4) swimming pool. Hand in your list one week from to-day

* For this section the drugs or other substances discussed are shown on the demonstration table or shelf. Identify them as you read, if not familiar to you.

2. Alcohol, 95 per cent. and 50 per cent.; each kills the colon bacterium in one minute. Why do most physicians prefer 50 per cent. for "the field of operation?" Which would have the most drying and fat removing action on the tissues, thereby delaying the healing process?

3. Hydrogen peroxide H_2O_2. This liberates oxygen, leaving only water. It is helpful in clearing out decaying tissue ("proud flesh"). To insure destruction of bacteria the H_2O_2 must come in contact with them. Therefore: (1) clean away from a wound all blood, pus, etc.; (2) add H_2O_2 in excess until the wound is clean. Too strong solutions must be avoided for mouth or throat gargles. The great objection to hydrogen peroxide is that it disintegrates on standing in the light, and it may be used with confidence even when it has lost all disinfecting value

4. Aniline dyes have a germicidal effect: (1) because of their chemical affinity for proteins (that is why they stain cell substances); and (2) because of the alcohol, carbolic acid, etc., used in making the stains (*e.g.*, carbol-fuchsin is usually 18 per cent. alcohol; the other 82 per cent. of it is a 5 per cent. solution of carbolic acid, making it still over 4 per cent. carbolic). Why do the stains used in the bacteriological laboratory remain sterile? See also p. 117

   Such stains as methylene blue and gentian violet are sometimes used in treating wounds; other aniline dyes are injected into the blood system to cure certain diseases, mainly protozoan. The ordinary staining dyes are not often used in medical practice.

5. Sulphur. Used mainly as a gas, H_2S, obtained by burning powdered sulphur or sulphur candles; 5 pounds per 1000 cu. ft. of space. Less used now than formerly, as it injures some colors and fabrics and has little penetrating power; more valuable as an insecticide than in killing micro-organisms

6. Chloroform, $CHCl_3$. 1 per cent. kills vegetative forms; no effect on spores. Useful mainly as an antiseptic on wounds, and in treating liquids (serum, anti-toxin, etc.) that cannot be sterilized by heat

   Iodoform (CHI_3). Not germicidal, but in decomposing tissue makes soluble iodine compounds that may (1) act on bacteria or (2) combine with poisons they have produced

7. Iodine. Surgeons differ greatly on the value of iodine. Some of its good points are: cheapness, ease with which it may be procured, small bulk, absence of the usual irritation in treated wounds unless applied too often. (More than once or twice a day on cut surfaces may dry the edges of the wound and

prevent healing.) (Iodine stains may easily be removed by applying starch.) Usually purchased in 10% or 15% strengths. There is possible danger in the recent popular interest in iodine as a water disinfectant (1 to 3 drops of iodine to a glass of water); this warning does not apply to iodine-treated water in "goitre belts"

8. Organic acids, such as lactic acid, are used because of their inhibiting or antiseptic values. Acetic acid has been described as having unexpectedly high germicidal value; even vinegar (10 to 15 per cent. acetic acid) affects some types of spores. Lactic acid has a well-known inhibiting effect upon many organisms (see p. 204); recall in this connection inhibiting effect or control of micro-organisms due to lactic acid in beer making

9. Boric acid, $B(OH)_3$. Commonly used, saturate solution, as an antiseptic. Valuable because non-irritating. While the other inorganic acids are strongly germicidal, they are too destructive (see p. 135) for general use. Boric acid combined with one or more essential oils is the basis of several patented washes for the mouth and teeth.

10. Essences or essential oils. Most of these have pleasant odors; an advantage? Explain
    (1) Thymol. Purchased as crystals or oil; most useful of these oils; diluted 1:1000 has antiseptic value; used for envelope moisteners, sponge dishes, etc.; usually rated one-fourth as strong as carbolic acid. Prominent flavor in listerine
    (2) Eucalyptol. Also one-fourth as strong as carbolic acid
    (3) Oil of cloves. Too sticky for the varied application of thymol; also too expensive. Often used in dentistry

11. Silver compounds
    (1) Argyrol. A silver and albumin compound which deteriorates rather rapidly. Its entire lack of caustic properties—one of its chief values—makes it difficult to be sure of its integrity. It is valuable for cuts, scratches, etc.; and some find it very helpful for sore throats. Apply (10-25 per cent.) with a brush. (Argyrol stains are easily removed with water or salt and water)
    (2) Silver nitrate. Too expensive for general use. Used for local treatment (infected eyes, throats) but too caustic to be handled by any except doctors or nurses. These wax ampules are issued by some state boards of health for treating the eyes of new-born infants

12. Other metallic salts or compounds
    (1) Corrosive sublimate (very poisonous); all such poisons should be colored with some non-irritating coloring to prevent accidental poisoning, *e.g.*, a few drops of methylene blue to a quart
        —Usually sold or made up in H_2O solutions (1:16 = saturate solution). 1:100,000, inhibits most bacteria; 1:50,000, kills many; and 1:1,000, good germicide
        —Alcoholic solution 1:500 in 95 per cent. alcohol. This is a little too "drying" for constant application, but excellent for securing initial aseptic conditions, as in cuts
    (2) Copper sulphate. Formerly popular as a disinfectant for water; now superseded by hypochlorites and chlorine (see No. 13 below; also water demonstration). 1:400,000 destroys typhoid in 24 hours; 1:1,000,000 destroys malodorous algæ (not uncommon in open reservoirs, late summer); not considered injurious to people in above strengths
13. Sodium compounds
    (1) Soap suds. Slight germicidal value; a cake of soap to a pail of water advised for disinfecting sick room (floors, woodwork)
    (2) Sodium carbonate (5 per cent.) kills vegetative bacteria in a few minutes
    (3) Sodium bicarbonate. No bactericidal effect; popularly used to counteract the acidity of the stomach or to prevent steam-sterilized instruments from rusting
    (4) Sodium hydroxide. Four per cent. kills resistant spores in one hour; too great action on human skin to be generally used
    (5) Sodium hypochlorite, calcium hypochlorite or a mixture of both, is used for disinfecting water, etc. Modified hypochlorites (plus sodium bicarbonate, boric acid, etc.) are valuable disinfectants for wounds; the deterioration in such hypochlorite solutions is unfortunately so rapid that such use is restricted to the hospitals and laboratories where they may be made up (and tested) every few days. Several firms are now advertising such chlorine or chloramine preparations. Look out for reports of tests for "keeping" qualities as well as the bactericidal efficiency of such patented preparations. (See p. 148)

14. Lime compounds. Valuable if used *fresh* and contact time (2 hours) observed
    (1) Lime or quick lime. Now obtainable in air-tight cans, can therefore be slaked (5 qts. water to quart can of lime) and used while fresh for disinfection (see (2) following)
    (2) Milk of lime. Fresh slaked lime diluted 4-8 times; useful when fresh (1 to 4 or 5 days old) to disinfect excreta and privy vaults
    (3) Chlorinated lime or "Chloride of lime." Really a hypochlorite (see 13 above). Purchasable in small tins at grocery stores; use on opening, diluting as directed by the label. (See p. 169)

15. Formalin. The disinfectants exhibited on this table are made of or combined with formalin. Formalin is rarely used as a liquid disinfectant; it is too caustic, and is "very hard on the hands." As a gas disinfectant, its practical value is equalled only by chlorine; it does not have the undesirable bleaching qualities of chlorine (*e.g.*, on clothing, upholstering)

    Advantages: little effect on fabrics and metals; a true deodorizer

    Disadvantages: very irritating to mucous membranes, and has but little effect upon insect pests, (making many doubt its bactericidal efficiency)

    The proportions for room disinfections are ten ounces of formalin to 1000 cu. ft. of air space. Cracks and crevices should be closed with pasted strips of paper, tightly packed cotton, etc., as in any other method of room disinfection

    Formalin tablets are said to give off enough formalin vapor to prevent bacteria from developing. Some recommend them highly at the beginning stage of sore throat. It seems very probable that sufficient formalin to make them effective would be irritating to the mucous membranes

    Efficiency readily increased by heat and by water vapor. All the methods for using formalin for room disinfection include some way of vaporizing it—
    (1) Allowing it to evaporate slowly from a wet surface, as a sheet, or, preferably,
    (2) Using heat
        (a) Burning formalin candles
        (b) Heating liquid formalin over a flame (gas, oil, etc.); this provides a higher temperature and gives a higher concentration of formalin in the room than (1)

(c) Heating the formalin by chemically generated heat

—by adding water and formalin to unslaked lime. The slaking of lime generates sufficient heat to vaporize the formalin

—by mixing paraformaldehyde (a powder form) with potassium permanganate and water

This combination acts slowly enough to give the operator time to leave the room. An added value of this combination is that it liberates O. The heat and the oxygen both add to the value of the disinfectant; and the vaporized water increases its penetrating power

16. Carbolic acid and the high coefficient disinfectants

(1) Crude carbolic, in liquid form, as purchased. Not easy to bring into true solution

(2) Diluted crude carbolic, less attractive than the pure, not suitable for treating dress goods, etc. (too oily), but used for utensils and excreta

(3) Pure carbolic crystals (C_6H_5OH) soluble in warm water; used for staining (mordant) and as a disinfectant. The efficiency is greatly increased by a little acid, such as HCl. Compute present cost per gallon of 5 per cent. solution. The recent European war has so affected the price of carbolic acid that substitutes are still in demand

Advantages of *carbolic:* does not destroy fabrics and colors; does not deteriorate; does not combine readily with albuminous substances (as excreta); efficient in low percentages; 1:400 kills most vegetative forms; and 1:20 kills most spores in a few hours

(4) Lysol. Twice as strong as carbolic

(5) Cresol ($C_6H_4OH_3OH$). Difficult to dissolve. Stronger than lysol; four times as strong as carbolic; often combined with potassium soaps; then twice as strong as carbolic

(6) Creolin. A cresol-soap combination. A cheap disinfectant; a dollar bottle makes 25-50 gallons of disinfectant

(7) Cresol, lysol, etc., are often called high-coefficient disinfectants. For example, lysol, as rated above (twice as strong as carbolic), would be given a coefficient of 2; cresol, of 4

(8) Other popular high-coefficient disinfectants: many of these are patented preparations (Neko, Hycol, CN, etc.). Calculate cost per gallon from cost and required strength as described on the label of one of these

(f) Other practical phases of disinfection

1. In a town (9000), two leading drug stores were asked to name the disinfectants they kept for sale, (1922). They had

1. "Chloride of lime"	8. formaldehyde
2. carbolic acid	9. hydrogen peroxide
3. mercuric chloride	10. sulphur
4. lead nitrate	11. calcium hypochlorite
5. copper sulphate	12. alcohol (denatured)
6. potassium permanganate	13. green soap
7. iodine	14. lysol

Do these cover the needs of ordinary living conditions? (See (e) on p. 142)
What situation, if any, is not met?

(g) Look over the recent pamphlets and articles on disinfection listed at the end of this chapter, selecting one for comparison with the information in this chapter

(h) Testing disinfectants

Advertisements of many patented, commercial disinfectants are sometimes misleading; *e.g.*, one reads, "Place a little of ———— in a dish on the radiator and avoid all disease." Obviously this would apply only to such disinfectants as liberate a germicidal gas or vapor, and which yield it in sufficient quantities to affect all disease organisms that might be distributed in the air of the room

Another direction, frequently met, substitutes "water box of the furnace" for the "dish on the radiator," thus claiming still more for the disinfectant

Select one of the disinfectants supplied by the instructor, or one which you think similarly misleading, and test it for its germicidal powers under the conditions described on the label.

Test it for vapor (or liquid) activity in some such way as illustrated in Fig. 97, using threads covered with a common non-spore-forming body organism, such as the colon bacterium (Fig. 75, II). To secure these, dip sterile threads (sterile forceps) into a fresh (24-hr.) culture, and place them in slightly open Petri dishes (Fig. 98) in the incubator, to hasten drying.

Then lower the thread into the tube of disinfectant as shown in Fig. 99. Leave it exposed to the vapor (or liquid) as directed on the label.

Then drop the thread into a fermentation tube of neutral red broth to see if live, vigorous colon organisms have survived, as indicated by apparent growth in the tube (p. 165). Watch for two or three days to see if (1) gas is formed and (2) if the color changes characteristic of *coli* also develop. Test the gas to see if both carbon dioxide and hydrogen are present, proving the presence of carbon dioxide by sodium hydroxide absorption (as in yeasts), and the presence of hydrogen in any remaining gas by the characteristic "pop" when the open end of the tube is brought to a flame (Fig. 76).

Has the disinfectant germicidal value, as shown by this experiment?

FIG. 97.—Method of testing a disinfectant for vapor effects. Most substances loosely advertised to "kill all disease germs if sprinkled around the room" fail to kill specific bacteria in such a test, being too diluted in such large amounts of air. (Ricker.)

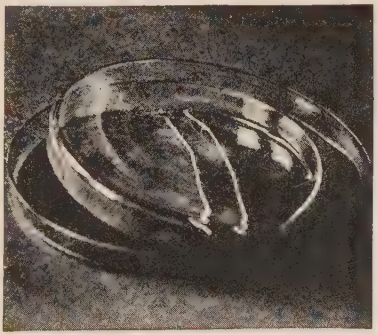


FIG. 98.—Threads drying in a Petri dish to insure that some of the organisms will adhere to the threads during the disinfectant test described under (h), page 148. (Ricker.)

FIG. 99.—Lower the dried thread into the disinfectant. Tilt or invert the tube to make sure that the thread is covered. Leave the upper end of the thread extending part way out of the tube to enable you to get it easily with the forceps; burn away the upper or exposed end before putting the thread into the broth.

How does this method of testing disinfectant value differ from those described in the pamphlets listed at the end of this chapter?

REFERENCES

(See also general reference list in Appendix)

Broadhurst and Holden: *Current Economic Bacteriology*, Williams and Wilkins, Baltimore. (Excerpts, Abstracts of Bacteriology. In press.)

Dakin and Dunham: *A Hand Book of Antiseptics*, Macmillan, New York, 1917.

Rideal and Rideal: *Chemical Disinfection and Sterilization*, Arnold, London, 1921.

PAMPHLETS

Anderson and McClintic: Method of Standardizing Disinfectants With and Without Organic Matter, U. S. Hyg. Lab., Bull. 82, 1912.

Ayers and Taylor: A Simple Sterilizer for Farm Dairy Utensils, U. S. D. A., Farm Bull. 748, 1916.

Beinhart: Steam Sterilization of Seed Beds for Tobacco and Other Crops, U. S. D. A., Farm Bull. 996, 1918.

Dorset: Some Common Disinfectants, U. S. D. A., Farm Bull. 345, 1914.

Hamilton: Disinfection, Parke, Davis & Co., Reprint 60, Detroit, 1914.

Hamilton: Facts and Fallacies in Disinfection, Parke, Davis & Co., Reprint 121, Detroit, 1917.

Hamilton and Ohne: Standardization of Disinfectants, Parke, Davis & Co., Reprint 45, Detroit, 1913.

Hilliard and Davis: The Germicidal Action of Freezing Temperatures upon Bacteria, Jour. of Bact., 1918.

Hite, Giddings and Weakley: The Effect of Pressure on Certain Micro-organisms Encountered in the Preservation of Fruits and Vegetables, W. Va. Exp. Sta., Bull. 146, Morgantown, 1914.

Houghton and Davis: A Study of the Germicidal Action of Ultra-violet Rays, Parke, Davis & Co., Reprint 56, Detroit, 1914.

McClintic: The Determination of the Phenol Coefficient of Some Commercial Disinfectants, U. S. Hyg. Lab., Bull. 82, 1912.

Pope: Methods of Disinfecting Stables, U. S. D. A., Farm Bull. 954, 1918.

Winslow and Abramson: The Effect of Drying Upon the Viability of Bacteria. Proc. Soc. Exp. Biol. and Med., 1912.

REVIEW AND STUDY QUESTIONS

1. Define disinfectant, germicide, deodorizer.
2. Contrast aseptic and antiseptic; inhibit and attenuate.
3. Name four different effects produced by physical or chemical agents that may lead to the death of the bacterial cell.
4. By what cell changes may the following cause the death of living cells: Electricity? Sunlight? High temperatures? Freezing? Salts?
5. Scan your list prepared for Exercise (e), p. 142: In how many instances could sunlight or boiling water be substituted?
6. Define concurrent disinfection; how does it affect the type of terminal disinfection which is necessary?
7. Give one use for each of the following: iodine; lime; formalin; calcium hypochlorite; alcohol; carbolic acid; soap suds and ozone.
8. What are high-coefficient disinfectants?
9. List the practical applications of steam pressure temperatures.
10. Why is moist heat a better disinfectant than dry heat?
11. Find (Current Economic Bacteriology) two or three new disinfectants which belong to the types or groups given in 7 or 8 above.

CHAPTER VII

AIR

LECTURE AND READING OUTLINE

A. Bacterial content affected by
   (A) Agents discussed in Chapter VI
      a. Drying. (See pp. 127 and 142.) Vegetative forms may die off at rate of 90 to 99 per cent. in a day. (See c below.)
      b. Sunlight
         (a) (For effects on bacteria see pp. 126 and 142)
         (b) Ultra-violet light valuable as stimulant to body; allows utilization to full of essentials for growth and health (with minimum vitamine supply)
      c. Temperature
         (a) (For freezing effects see pp. 133 and 158.)
         (b) Alternation of extremes destructive to bacteria. Lower bacterial content of air at high altitudes (tuberculosis resorts) related to abrupt night and day changes in temperature as well as rapid drying of organisms
   (B) Suspended particles (dust, lint), acting as floaters for bacteria
      a. Related to bacteria in hailstones
      b. Proportion low, 1 bacterium to 30 or 40 million dust particles
   (C) Number of inhabitants affects rate at which bacteria are added to air, *e.g.,* by nose and throat spray
   (D) Water surface exposed
      a. Wet surfaces hold dust and bacteria brought into contact; explains low content of sewer air
      b. Washing effect of rain and snow decreases air content

(E) Altitude

a. Pasteur's open flask experiments showed lower content for Alps than Paris; temperature, etc., also factor here; see b. following

b. Counts per cubic meter of air range from zero on mountain tops to many thousands in city streets. Such differences, a combination of other factors: temperature, population, light

B. Methods of estimating bacterial content based on number of colonies developing on agar plates. Such bacterial counts relative, not absolute, as food materials and temperatures used not favorable for growth of all bacteria

(A) Exposure of agar plates

a. Accuracy affected by air currents, suspended particles

b. Not considered reliable index of actual content

(B) Washing a given bulk of air, *e.g.*, forcing it through water; the washings are sedimented or centrifuged, and the sediment used to make agar plates

C. Ventilation problems

(A) Temperature and moisture usually main problems

a. Directly, affecting comfort; rate at which body loses heat

b. Indirectly

(a) Extremes, predisposing factors in disease

(b) Drafts. Sudden or great moisture and temperature may affect nasal membranes (size, blood supply) and aid bacterial invasion of such areas

(B) Bacterial problems usually secondary

a. Note decrease of diseases now cited as air-borne; compare transfer methods, in the various diseases (U. S. P. H. S. pamphlet, No. 436), noting greater importance of direct contact

b. In crowded places (street cars), air transfer becomes practically direct contact; important in epidemics of respiratory diseases, where air is overcharged

DEMONSTRATION AND LABORATORY WORK

1. Photographs of three agar plates (Fig. 100) each exposed for the same length of time (5 min.), following dusting operations on the same day; made in the following order: (1), damp

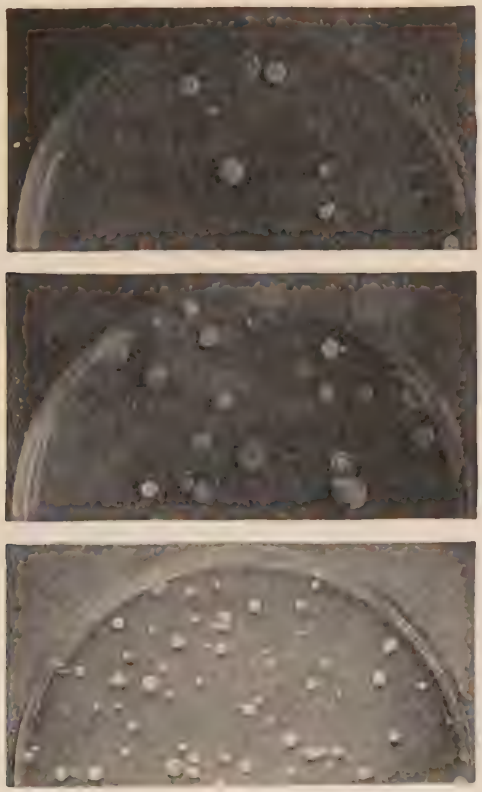


Fig. 100.—Agar plates exposed in the same room as described in Exercise 1, on this page. (Ricker.)

cloth; (2), dry cloth; (3), feather duster. This shows in general the differences one would expect, but such experiments are very uncertain tests of air content.

Is it possible to have the conditions in a given room exactly the same except for the method of dusting? Would such air plates made in different rooms be at all comparable? (See p. 155.)

2. Two types of apparatus (Fig. 101) for making bacterial counts of air content. Which is the simpler? Which seems likely to give more uniform results? Note that in these, every

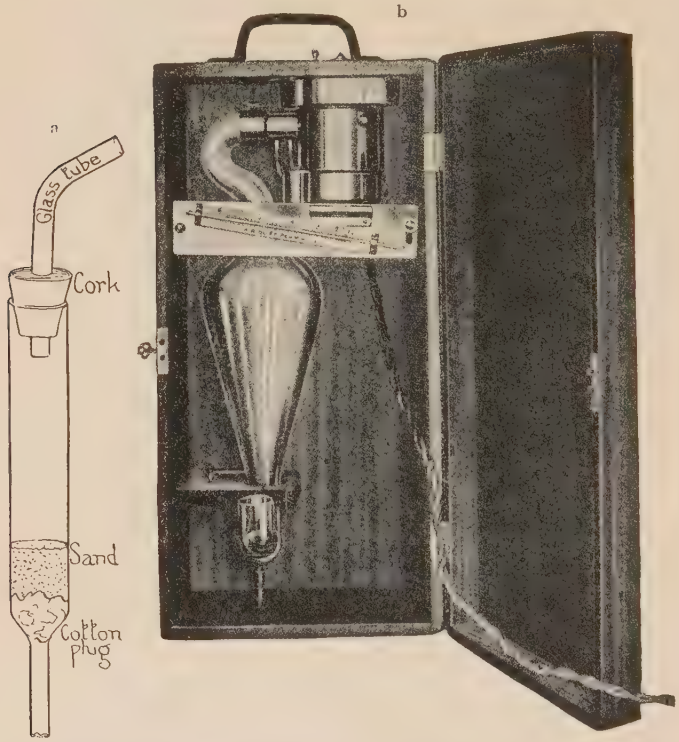


Fig. 101.—Two types of apparatus used for separating bacteria from the air as it is drawn or pumped through wet sand or water.

In a, on the left, the wet sand is washed in water and that water is used to make agar plates; the relative numbers of bacteria developing per cu. ft. or cu. yd. of air indicate the degree of air pollution.

b. A little motor in the upper part of this apparatus draws the air through the water in the upturned tube at the bottom of the large glass bulb. The water spray forming in the upper part of the bulb helps wash the air. The rate (amount of air being washed) is registered on a scale extending across the box. (Wallace and Tiernan.)

one of the bacteria in a given volume of air may be caught or held in the water used to make the plates. In such tests as No. 1 above, only the bacteria that happen to fall out of the air present or passing over each plate could be represented on it.

3. Are air-exposed plates reliable indicators of air conditions?

   (a) Melt a tube of agar and pour it (with aseptic precautions, as shown by the instructor) into a sterile Petri dish. (If the agar does not cover the whole plate, move it horizontally back and forth, while it is resting on the table, to spread it evenly. Do not lift it from the table to accomplish this. Why?)

   (b) When the agar has fully solidified or "set," (in 30-40 min.), expose the agar surface for three minutes in the place assigned to you (telephone booth, street corner, swimming pool, etc.)

   Arrange with fellow students to include several paired situations: a room before and during dusting; near a closed and an open elevator shaft; or outdoors and indoors at the same level.

   Replace the cover promptly and place your exposed plate in the tray provided.
   It will be incubated at 37° C. for two days, and at room temperature for the rest of the time elapsing before your next class hour. What advantage in using both temperatures?

   (c) Count and record the colonies which develop during the incubation.

   (d) Compare your results with those obtained by the other students. Do they agree with your general impression of the various localities? Do they support the statement that (1) air movement, (2) dust and lint content of air and (3) water surface exposed are important factors affecting the bacterial count of air? Is this a reliable method of testing air? How does it differ from the standard methods described in the references provided by the instructor?

4. Look through the pamphlets and books listed at the end

of the chapter and enrich your reading outline by material on the assigned number of the following topics or questions

(1) What ranges in bacterial content have been reported for indoor air? For outdoor air?

(2) What range in bacterial content has been reported for dust: (a) house sweepings and (b) street dust?

(3) Look through the references dealing with special conditions (in churches, schools, hospitals, etc.). Can you find any criticisms, standards, or recommendations which relate to bacterial content directly?

(4) In the above, what recommendations are beneficial because of indirect effects on bacterial content?

(See general reference list in Appendix)

PAMPHLETS

Bolton: The Problem of City Dust, Report, Am. Soc. Heat. and Vent. Eng., 1915.

Winslow and Browne: The Microbic Content of Indoor and Outdoor Air, Monthly Weather Review, 1914.

Winslow and Kliger: A Quantitative Study of the Bacteria in City Dust With Special Reference to Intestinal and Buccal Forms, Am. Jour. Pub. Health, 1912.

REVIEW AND STUDY QUESTIONS

1. What are the normal air conditions or qualities that affect the survival of air bacteria?
2. Justify bacteriologically the housewife's warfare upon dust.
3. Describe the process of making a bacterial count of room air.
4. Give representative air counts for various localities and explain the wide differences possible.

CHAPTER VIII

WATER

LECTURE AND READING OUTLINE

A. Relation to disease
   (A) Water-borne diseases: typhoid, cholera, dysentery; relation of water to tuberculosis not so easily shown (no definite incubation period)
   (B) General health: Mills-Reincke theory, relating drop in diseases not water-borne (*e.g.,* respiratory) to improved water supply

B. Origin of water bacteria
   (A) Air; number related to winds, washing effect of rain and snow falling into water
   (B) Animal excretions, including sewage, reaching water
   (C) Soil
      a. Soil as a filter
         (a) Upper 6 inches richest bacterial flora
         (b) Below 4–5 feet, soil quite free from bacteria
         (c) Filtering character eliminates danger from burial grounds
      b. Surface contributions
         (a) Plant and animal decay; affect bacterial content indirectly, also, by supplying food
         (b) Surface washings; effects marked
            1. Manured fields
            2. Polluted water sheds
      c. Persistence of organisms
         (a) Vegetative stages, sometimes surprising: *e.g.,* typhoid, several months
         (b) Spores may persist for years; anthrax and tetanus have survival records of ten years or more

d. Control of water pollution through soil
   (a) Relative position of barns, outhouses, etc., important; surface slope may allow drainage into water
   (b) Character and slant of underground layers; wells, etc., may receive practically unfiltered water running along impervious layer (clay) or through underground channels (limestone)

C. Bacteria in ice
   (A) Effect of freezing
      a. Rate, 50 to 70 per cent. killed in freezing process; decrease continues with storage: 90–99 per cent. killed in 2–4 weeks
      b. Typhoid
         (a) High percentage also killed by freezing
         (b) "Winter typhoid" explained by greater resistance of typhoid bacteria surviving freezing in polluted water; inactive or resting condition at low temperatures
   (B) Comparison of natural and artificial ice
      a. Natural ice
         (a) Usually stored several months
         (b) Low percentage of original water bacteria survives
         (c) Original content related to type of water (polluted) and method of cutting (manure, horse used with ice saw)
      b. Artificial ice
         (a) In closed tank method the water is boiled to eliminate air bubbles and sediment; always safe
         (b) Open tank method, water not necessarily boiled
         (c) Storage period usually lacking; safety therefore depends on quality of water and process

D. Methods of improving water supply

(A) Predatory organisms, such as protozoa and small crustacea; difficult to measure or secure definite estimates

(B) Storage

a. Sedimentation (gravity an indirect factor, see p. 124)

b. Exhaustion of food supply; storage periods (thirty to forty-five days) often used successfully in community supplies without other methods

(C) Light

a. Natural sunlight, germicidal action limited by

(a) Depth; may extend 9 to 10 feet

(b) Turbidity (silt)

(c) Rate of flow or time exposed to light

b. Ultra-violet light (see p. 126)

(a) Modified also by salts in solution

(b) Practical applications

1. Satisfactory for small amounts: boat supplies (lake steamers) and swimming pools.

2. For town supplies; demands higher type supervision than other community methods; not yet widely used

(D) Oxidation

a. Natural, due to air contact (see oxygen, p. 123); limited by lessened free oxygen supply in polluted waters, due to affinity of polluting organic matter for oxygen

b. Oxygen treatment (see p. 162)

(E) Dilution

a. Often relied upon

(a) City water supplies; sewage. Polluted supply (river) diluted by later tributaries less polluted

(b) Swimming pools; constant inflow of unused water

b. Aided by effects of light, aëration, etc.

c. Must take time into consideration, *e.g.,* hourly rate of occupancy in swimming pools, as well as actual dilution rate

(F) Filtration

a. Soil as natural filter, as in wells (see p. 157)

b. Artificial filters

(a) Community filters

1. Rapid sand filters

(1) Mechanical filtering action only

(2) Coagulants necessary; size of bacterial masses increased and therefore caught between sand grains

(3) Rate high; 50 to 100 times rate of slow sand filter

2. Slow sand filters

(1) Action

—only partly mechanical.

—sand depth itself (4 feet)

—gelatinous film on upper surface (plant and animal debris; precipitate due to coagulant)

—mainly bacterial; due to oxidizing bacteria, present as film on the sand grains; these oxidize organic matter, destroying bacteria originally present and their food materials

(2) Rate: about a million gallons per acre of surface

(b) House filters

1. Even best types usable for only short periods before becoming clogged, slowing action and allowing bacteria to go through
2. Flow always slow; too slow for usual house demands
3. Boiling is preferable for emergency use
4. Cotton or paper pad-filters preferred to remove silt; such pads should be renewed at least twice daily
5. Charcoal absorbs color, etc., but not advisable as filter (animal charcoal may favor bacterial growth)

(G) Heat

a. Boiling

(a) Death point of ordinary pathogenic bacteria 80° C., therefore boiling insures protection

(b) Subsequent aëration to make palatable replacing air

(c) Rarely used for large supplies; chemicals substituted

b. Application of lower temperature for large supplies (pasteurization)

(a) Advantages; cheap, and continuous application possible (waste heat in pumping station, chimney might be utilized)

(H) Distillation

a. Bacterial content lower than boiled water

(a) Mineral content lower; flat in taste

1. Minerals added to distilled water for drinking
2. Aëration for taste, sparkle

(I) Chemical agents

a. Natural agents

(a) Light (already discussed)

(b) Oxygen, amount of broken surface (brooks, waterfalls) important; popular misconceptions concerning "self-purification"; "safe in 200 ft. flow", etc.; compare river-carried typhoid, *e.g.*, Lowell to Lawrence, though 9 miles apart. (See ozone below.)

b. Artificial methods

(a) Copper sulphate. Effective against bacteria; also against algæ, which often cause alarm by production of disagreeable odors and tastes

(b) Hypochlorites. Often confused with chlorides, which are more stable, and not active liberators of oxygen and chlorine (see p. 169). Widely used

(c) Chlorine. Constantly gaining in use; amount more easily regulated than with hypochlorites.

(d) Ozone. Unstable and more active form of oxygen. Excess sterilizing amounts not so objectionable as with chlorine or hypochlorites, especially in swimming pools

(e) Ultraviolet rays (see pp. 126–7)

E. Examination of water

(A) Bacterial count determined by plating small amounts (one c.c.; usually less)

a. Number in drinking water low, since ordinary water contains little organic material

(a) Unless excess organic matter present

(b) Unless pollution recent

b. Accepted standards

(a) Spring, well, very low; 100–200 per c.c. the upper limit expected

(b) River water containing 1000 per c.c. unsafe unless treated

c. Importance of method of taking sample for test

(a) Size of sample

(b) Depth, distance from shore, sewer, outlets, etc.

(c) Age and care of sample; bacteria may decrease or increase several thousand times on standing a day
(d) Both source and history of supply must always be taken into consideration, as well as bacterial count

(B) Kind more important than number
a. Kind determined by
(a) Search for pathogenic organisms themselves, by use of selective medium
1. Typhoid: brilliant green dye in agar (Fig. 81, II) allows typhoid to develop, but inhibits other intestinal forms
2. Cholera: high percentage of peptone favorable for growth of this intestinal organism
(b) Demonstration of accompanying organism, *e.g.,* from fecal sources
1. Colon bacterium, constant inhabitant of intestines of man and other vertebrates shown: (1) by color changes in media containing lactose and neutral-red dye (see p. 165); (2) by gas (both CO_2 and H) from lactose

(C) Chemical tests
a. Based mainly on organic matter content
b. Tests
(a) Amount of oxygen absorbed; directly related to organic matter present
(b) Tests for simple nitrogen compounds (ammonia, nitrites,) which indicate presence of decomposing nitrogenous substances

DEMONSTRATION AND LABORATORY WORK

1. Organisms associated with undesirable or dangerous water conditions
a. *Streptococcus,* a long-chained form (Fig. 28, II). (Some are always short-chained, 4–8 organisms only.) Long-chained ones are probably never found in water free from animal pollution; at our latitude, they are not common even in polluted waters

b. *Bacterium typhosus.* Notice the wide apparent range in size (Fig. 41, II). This is partly due to the fact that some of the organisms remain connected after division, forming filaments. (See Fig. 65.)

c. Colon bacterium. Could you be sure of distinguishing between these (Fig. 75, II) and the typhoid organisms?

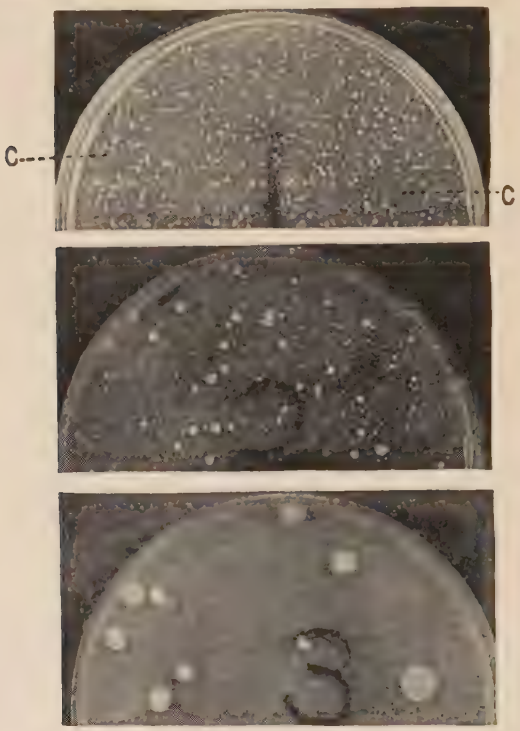


FIG. 102.—These three plates represent respectively 1 c.c., 1/10, and 1/100 c.c. of tap water. (Read down.)

Often found in water; almost invariably present in feces and sewage, and therefore important in indicating possible dangers in a given water supply. Would there be danger of typhoid in water which did not contain *coli*?

d. *Vibrio comma* or cholera organisms (Fig. 108, II). Short, slightly curved organisms, often comma-shaped.

e. Dysentery organisms (Fig. 52, II); like typhoid, not easily distinguished microscopically from other rods.

2. Water examination.

a. Plates made from tap water (1 c.c. and 1/100 c.c. to a plate). Are the respective colony counts (Fig. 102) what you would expect? Explain the generally larger size of the colonies in the weaker dilutions.

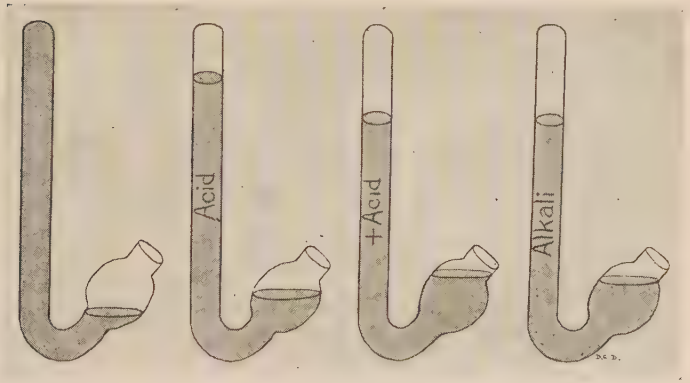


Fig. 103.—This reddish neutral red broth turns more cherry colored as acids accumulate; yellow indicates a preponderance of alkali. Do the changes in the *coli* tubes shown in the laboratory agree with the labels of the last three tubes pictured here? (Only tubes with plugs would be used for such tests, of course). To answer Exercise F, on p. 166, compare the tube provided by the instructor for that situation with these results. (See also Fig. 76.)

What does a large count indicate? Why do we not depend on number only in judging water?

b. Some organisms are tested for by presumptive tests. Bile, neutral-red, brilliant green, lactose, and litmus are among the special substances used. Note the action of *coli* on these litmus-lactose-agar plates. Why do we examine for the comparatively harmless *coli* organism instead of typhoid? Are we justified in condemning water on positive *coli* tests?

c. Plates of three dilutions (1:10; 1:100; 1:1000) of a sample of water on lactose-litmus-agar plates; these show *coli* in the 1/10 dilution plate only. What does this indicate concerning the condition of the water? In

New York City 10 to 50 *coli* colonies per c.c. are allowed in oyster tests; not more than 10 in drinking water. Does this give any protection to the consumer?

d. These tubes (Fig. 103) contain *coli* growing in neutral-red and lactose broth for 1, 2 and 3 days respectively. What two changes does *coli* produce in this medium? How do the reaction changes vary with time?

e. The tubes provided are three-day cultures of *coli*. Test the gas with sodium hydroxide (p. 66) to see how much of it is carbon dioxide.

Bring any remaining gas to the open end of the tube (Fig. 76), and test it for hydrogen. (*Coli* is not ordinarily disease-producing; it is advisable, however, to disinfect the hands after this experiment. Work over a jar of disinfectant, and drop the tube in it, when the test is completed.)

f. Material (milk, oyster, water) from the source indicated by the accompanying label was used to inoculate neutral-red and lactose broth. Is *coli* present?

g. Brilliant green has a decided inhibitive effect upon most intestinal organisms, but allows good development of the typhoid organism. Explain the proportion of colon and typhoid colonies in the plates containing two different strengths of brilliant green dye (Figs. 81 and 82, II).

WARNING: Do not hold typhoid plates so as to get any of the condensation water on your hands. What should be done with hands, tables, etc., accidentally so moistened?

h. *The Standard Methods of Water Analysis* is the book followed by the leading laboratories in the country. It includes:

(1) Collecting water samples.
(2) Physical examination: color, sediment, etc.
(3) Chemical examination.
(4) Bacteriological examination, and a description of the common water bacteria.

Glance through it to get a general idea of these

four parts. What medium new to you is considered helpful in testing water? Is the emphasis on bacterial counts? On presumptive tests?

3. House filters.

a. A Berkefeld filter (Fig. 50, II) unscrewed to show the various parts. A piece of broken filter candle (Fig. 51, II) shows the porous character of these "porcelain" filters. How many possible places for leakage? How may such leakage be prevented?

Fig. 104.—A common kitchen sieve lined with a single layer of absorbent cotton. The cotton should be renewed every morning, at least. Such filters are uncertain, and in emergencies, when such domestic treatment is necessary, water should be boiled to kill possible disease organisms, the cotton filters being used merely to remove fine earthy material, etc. (Ricker.)

b. Emergency filters to remove sediment; bacterial safety should be insured by boiling the water rather than by using house filters. (See p. 161.)

(1) A home-made cotton filter (Fig. 104) with a wire support for the cotton pad.

(2) Cheap commercial paper pulp and cotton filters. The filter pads should be replaced at least twice a day. Why?

c. Bacterial counts to test the efficiency of household filters.

(1) Collection of samples to be tested.

(1) One to several hours before the class session, place on a tap one of the house filters used in your locality, or one of the small standard laboratory filters (Berkefeld, Chamberland, etc.). At the beginning of the class period, collect in sterile test tubes (as shown) a sample of the filtered water, and a sample of unfiltered tap water, from a similar tap (or, if necessary, remove the filter after collecting the filtered sample).

(2) Make 1/10 dilutions of each sample (as shown by the instructor), and use one c.c. of each dilution to make an agar plate. (Cool the agar tubes to 45°–47° C. before adding the cubic centimeter of water.) (Why?)
Incubate the plates for two or three days at 37° C., and then count the bacterial colonies present on each plate. Use a hand lens, and count, preferably, by looking at the bottom of the lower dish. (Why?) Record the count per cubic centimeter (10 × the actual plate count) for each sample.

(3) After the filter has been in use three to five days, repeat this exercise, or arrange to have another section of the class make similar plate counts, before changing the filter-pad (or "candle").

(4) Did the filter lower the bacterial count? Is it desirable for home use?

4. Chemical treatment of water.

a. What substances for rendering drinking water safe were given in the demonstration on Effects of Physical and Chemical Agents? (P. 142.)

b. Hypochlorites.
Sodium and calcium hypochlorites are now used in water disinfection. The usual proportions for bleach-

ing powder or calcium hypochlorite are 1 or 1½ parts of available chlorine to a million parts of water.

(a) These hypochlorites so widely advertised to-day are not chlorides
Calcium chloride is CaC_2, but calcium hypochlorite is $CaCl_2O_2$. It has O to liberate, which gives it added disinfecting (oxidizing) value

(b) Chlorine is also liberated and adds to the disinfecting power of the hypochlorite. HOCl is present, as hypochlorous acid, which liberates O also, forming HCl which is a disinfectant, though usually too small in amount in such cases to be of real value

c. Chlorine. Look through the references listed at the end of the chapter, and find supporting arguments or evidence for chlorine (1) as an emergency water disinfectant; (2) as a routine disinfectant instead of hypochlorites.

d. Look in the same reference list for reports of successful results with (1) ultra-violet rays; (2) with ozone.

e. What is there in common in the problem of water purification and the problem of sewage disposal?

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(See also general reference list in Appendix)

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REVIEW AND STUDY QUESTIONS

1. What are the sources of water pollution?
2. How is ice related to disease transfer?
3. List the methods of improving community water supplies, with a ten to twenty word characterization of each method.
4. What chemical agents are used in purifying water?
5. How may we determine the number of bacteria present in water? Why are *numbers* of interest?
6. How may the safety of water be demonstrated? Why is bacterial investigation limited to intestinal types?
7. What is the *coli* test? Why is it used?
8. Give rulings concerning house filters.
9. Why are sand filters approved for community supplies while small (porcelain, charcoal, clay) filters are not approved for home use? Why are these same clay or porcelain filters indispensable for bacteriological elimination in laboratory work?
10. What are the prominent phases of water bacteriology in any board of health laboratory?

CHAPTER IX

MILK

LECTURE AND READING OUTLINE

A. Milk-borne diseases
   (A) Connection established (epidemics, carriers, etc.) for typhoid, diphtheria, scarlet fever, tuberculosis (both human and bovine), septic sore throat, foot and mouth disease, and infections due to streptococcus and staphylococcus bacteria
   (B) Diseases less positively traced to milk: mumps, measles, whooping cough, chickenpox, and smallpox
B. Milk as obtained from healthy cows
   (A) Food value high
      a. Rich in needful nutritive substances
      b. Foods in soluble form
   (B) Bacteria at time of milking
      a. Fore-milk (milk held in teat canal) rich in bacteria
         (a) Teats in frequent contact with ground, etc.
         (b) Moist tips of teats favor growth of chance-contact organisms
      b. Udder diseased: tuberculosis, streptococcus infections, etc.
         (a) Pyogenic invaders may persist for months or years
         (b) Disinfection or other destruction of organisms adhering to labyrinthine cavities of udder practically impossible
         (c) Adjacent lymph glands often real seat of infection
      c. Opportunities affording entrance of bacteria into milk

(a) Cow: saliva, coat (dust, manure), moist teats, tail (commonly soiled, switching during milking)
(b) Barn dust, fecal material in stalls, filthy barnyards
(c) Air currents during milking
(d) Milker
1. Unwashed hands; saliva of milker, especially in "wet milking"
2. Clothing soiled by other barn work
(e) Pails
1. Incompletely cleaned (seams)
2. Size of open top important
(f) Small animals allowed in stables

(C) Possible freedom from micro-organisms
a. Mid-milk relatively free, at least in healthy cows
b. Low count of samples milked with aseptic precautions
(a) Raw milk and cream sent to Paris Exposition, 1900, served sweet when 10 to 14 days old
(b) Low bacterial count and high keeping qualities of special milks supplied by certain dairies for baby diets, steamer gifts

C. Great increase in bacterial content of milk with age
(A) Growth conditions
a. Foods in milk
(a) All nutritive groups represented in milk; adapted to wide range in bacterial species obtaining entrance to milk
(b) Suitable proportions
(c) In solution (ready for absorption)
b. Water present; necessary in all metabolic processes
c. Range of favorable temperatures as milk cools; should be cooled quickly to shorten period at more favorable upper range of temperatures

D. Control of the flora of milk
  (A) Control of bacterial content slows multiplication of
    a. Pathogenic organisms, such as dysentery, typhoid
    b. Non-pathogenic organisms causing undesirable changes in milk
      (a) Souring; affects appetizing quality
      (b) Actual decrease in food values
      (c) Other undesirable substances: tastes and odors (bitter, "stale"; buttery, fishy, etc.)
  (B) Methods of affecting the bacterial content
    a. Low temperature
      (a) Effect on growth of bacteria
        1. Bactericidal action of substances normally present in fresh milk decreases count when kept 32° to 40° F.
        2. Inhibition of growth by low temperatures. 50° F. reasonable upper limit for iceboxes and refrigerator cars; preferably lower
      (b) Enforced refrigeration of milk supplies
        1. Necessary, especially during train transit to city; whole time, cow to consumer, often two or more days
        2. Desirable changes: extend refrigeration
          (1) To farm itself; where often kept 12 to 18 hours without cooling, before delivery to collecting station
          (2) To home; temperature on house delivery and in home usually too high; should be kept in ice chamber itself
    b. Chemicals; prohibited because
      (a) May be injurious, especially when milk is main article of diet, as with babies
      (b) Mask real quality of milk; cover for lack of cleanliness

c. Heating

(a) Pasteurizing

1. So-called, because like Pasteur's method of affecting bacterial flora of wines
2. Temperatures advised: 62.5° to 65° C. (145° to 150° F.) for 20 to 30 minutes; 45 minutes recommended by some. "Flash" processes: 80° C. for one minute not adapted to large bulks; uniform temperature not certainly obtained
3. "Capping" by machine, after pasteurization, advised; no chance for human contamination
4. Effect upon bacteria
   (1) Kills common pathogenic bacteria, even tuberculosis
   (2) Does not kill spores; many common protein-breaking organisms form spores; these may therefore be predominating organisms after pasteurization, especially in milk containing manure or other intestinal material rich in such organisms
5. Arguments against pasteurization
   (1) Cannot remove dirt, odors and accumulated bacterial wastes
   (2) Delays souring, and is therefore a cloak for poor grade of milk
   (3) Permits careless methods of milking or handling preceding pasteurization; partly met by limiting bacterial content at time of pasteurization as in New York City regulations

(4) Induces nutritional diseases (malnutrition, scurvy); not substantiated. Reports of city milk stations do not show increase but decrease; tables on baby feeding show higher weekly gain for babies fed on pasteurized milk than on unpasteurized of same quality; disease less (cases, days of illness and deaths) with pasteurized than same quality raw milk

(5) Destroys vitamines; replaced by fruit juices (orange or tomato) which have minerals making them valuable additions to the diet

(b) Higher temperatures (boiling)

1. Cause unnecessary loss in normal vitamines and enzymes
2. Temperatures above pasteurization unnecessary for killing ordinary pathogenic organisms (tuberculosis)
3. Useful in emergencies, *e.g.*, for suspected milk when lacking pasteurization equipment

E. Bacterial standards

(A) Lowest count demanded, certified milk: 10,000 per c.c. on house delivery

(B) Community standards rarely as low as 20,000 to 30,000 for ordinary milk supply. Advised standards (Am. Med. Assn.):

a. Grade A, 10,000 on house delivery (raw or pasteurized)

b. Grade B, 50,000 on house delivery (pasteurized only)

(C) Standards in relation to pasteurization

a. Pre-pasteurization counts related to food quality of milk (amount of bacterial destruction of food): limit (500,000 per c.c.) on count before pasteurizing

b. Count after pasteurization affects keeping period as well as food quality

F. Methods of examining and controlling milk supplies

(A) Bacterial count

a. Ordinary agar plate method (see pp. 181–2)

b. Frost's little plate method

(a) Milk added to small amount of agar on slide; then incubated, stained and count made

(b) Stained slides serve as permanent records

c. Direct examination or count

(a) Advantage to consumer; quality determined before milk consumed

(b) Demands more skilled workers than plate method

(c) Relationship to plate count, 4 to 8 × the plate count, explained partly by

1. Clump *versus* single bacterium counts
2. Dead bacteria (pasteurized milk) may take stains; counts higher than where count depends on growth of organisms (a and b above)

(d) May show disease

1. Bacteria, if form characteristic, as streptococci
2. Leucocyte excess may indicate diseased conditions (pus in udder, etc.)

(B) Presumptive tests—special media as in testing water for *coli* (pp. 102 and 165) to determine presence of intestinal organisms and probable danger of typhoid, dysentery

(C) Inoculation tests (guinea-pig) for pathogenicity; used to obtain legal evidence if illness (*e.g.*, tuberculosis) thought traceable to given milk supply. Not used to protect; rather, to prosecute

(D) Cleanliness tests

a. Sieve method, Park and Schroeder sieve

b. "Dirt" (manure, hair, etc.) associated with high counts

c. Milk obviously dirty cannot be clean milk (though, unfortunately, apparently clean milk is not necessarily clean)

(E) Score cards

a. Score or total count based on

(a) Cow: health, cleanliness
(b) Stable conditions
(c) Workers or dairymen
(d) Cleanliness tests of milk
(e) May include bacterial record
(f) Fat percentage

b. Price paid farmer may vary with milk quality, as summed up on score card, as well as with fat test

G. Milk products

(A) Evaporated milks

a. Dried milk

(a) Quality more a question of original substance evaporated (whole milk, skim milk) than of bacterial content
(b) Changes, rancidity, etc., relatively slight

(B) Fermented milks

a. Alcoholic: koumiss; formerly made from mare's milk; now a yeast and bacterial fermentation product from ordinary milk

b. Acid (lactic)

(a) Ordinary souring organisms checked when acidity reaches one per cent.
(b) Organisms producing higher acidity (up to four per cent.)

1. Commonly cultivated types

(1) *Lactobacillus bulgaricus* (Fig. 18, II) of Metchnikoff; used in several types of soured milk, artificial buttermilks, etc., limits growth of protein-splitting organisms; usually overgrows and inhibits pathogenic organisms; typhoid, etc., rarely traced to soured milks
(2) *Lactobacillus acidophilus* (Fig. 17, II); equally active in milk cultures; preferable to

bulgaricus as supplanting organism in intestinal complications; it adapts itself more readily to intestinal conditions; may persist as prominent inhabitant

2. Home preparation of milk drinks
   (1) Inoculation of desired organism, using soured milk (teaspoonful), broth culture (whole tube as purchased) or tablet (dried organisms). Large amounts for "inoculate" decrease probability of persistence of original milk organisms
   (2) Incubation, about 30° to 37° C., warm part of kitchen, etc., for 24–48 hours
   (3) Mellowing in icebox, two to three days to soften curd
3. Helpful results
   (1) Partly due often to improved quality, and sometimes increased quantity of food supply, when patient is given complete milk diet
   (2) Mainly due to inhibition or complete replacement of intestinal flora by *Lactobacillus acidophilus;* often cures intestinal disturbances (auto-intoxication) due to activity of undesirable organisms, particularly protein-splitting forms preferring an alkaline to an acid environment
   (3) Diet changes—additions of lactose, dextrin, or even plain milk in sufficient quantities

may cause complete replacement by *acidophilus,* apparently common resident of intestine

(C) Butter

a. Rarely carrier of disease, even tuberculosis; explained by
   (a) Preliminary souring process which is inimical to most disease organisms
   (b) Preliminary pasteurizing process, now common commercial method of butter-making, to reduce spoilage organisms
   (c) Salt content: 4–5 per cent. salt with 14–15 per cent. H_2O means a bacterial environment of high salt concentration
   (d) Storage period, often covering months

b. Use of starters: more uniformly good products of creameries due to use of "starters," mixtures of desirable organisms (flavor, texture); their action (acids) unfavorable to chance organisms producing bitter, ropy, slimy, blue, "bloody," "sweet curdling," or other undesirable changes, sometimes occurring in milk in home butter-making

(D) Cheese: kinds numerous, varying with

a. Original substance; whole milk, skim milk, goat milk, additions of cream, bread, sage, etc.

b. Types and sequence of organisms: both important in effect upon texture and flavor
   (a) Types include not only bacteria but yeasts and many other molds; some in other food-stuffs commonly considered spoilage organisms, such as *Penicillium* or blue mold, used in Roquefort cheese
   (b) Sequence—may result in entire disappearance of initial type or types; may extend through weeks or months

(c) Cheese made of known organisms; now possible with several different cheeses, such as Swiss, Roquefort, cream, and Cheddar. Cheddar cheese of good commercial value has been made with a single organism, a species of streptococcus (Fig. 184)

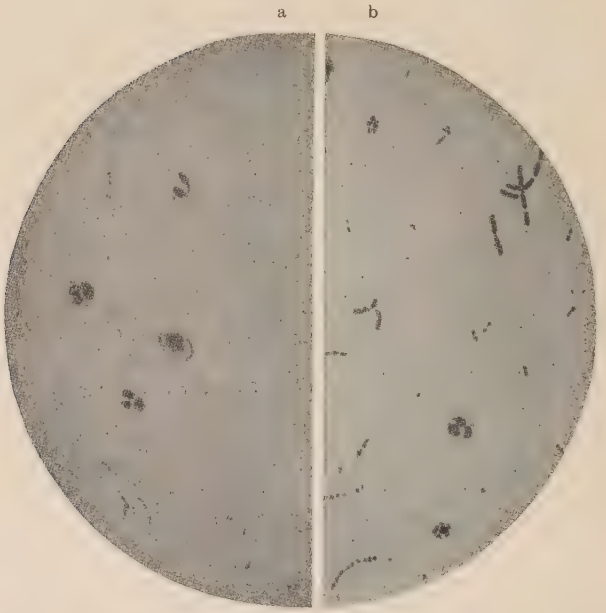

Fig. 105 a.—Part of a slide for the direct examination of the bacteria present in milk. An average of one bacterium to a slide (with the ordinary oil immersion lens) would mean over 600,000 bacteria to a cubic centimeter, or about 40,000 to a drop of milk.

b. If the diameter of this field is .2 of a millimeter, and there are 6366 such fields on the whole smear on the slide made from 1/100 c.c. of milk, how many bacteria are there in a c.c. of this milk?

DEMONSTRATION AND LABORATORY WORK

1. Milk examination

(1) Microscope slide of raw milk of good quality (stained with methylene blue). No bacteria are present in this field (Fig. 105, a). In the center, note white cor-

puscles (leucocytes) with two or more nuclei. Too many corpuscles are a sign of inflammation, and probably indicate diseased conditions in the udder or the nearby lymph nodes. Authorities differ about the number to be allowed with safety in milk. The numerous round unstained areas of varying size, often seen in such slides, are due to the oil globules present

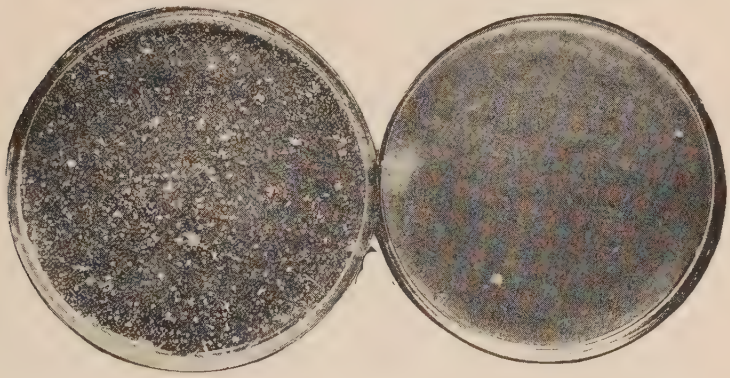

Fig. 106.—The same milk: raw (left) and pasteurized (right); each plate contains 1/100 of a c.c. of milk. In the pasteurized plate there are 24 colonies (one mold on left margin more or less distinctly shown). Was the milk originally of good quality? (Ricker.)

in the milk. Compare this slide with the one you have made (p. 64), in which you removed the fat first. Why is fat removal necessary for accurate microscopic work?

(2) Another smear (Fig. 105, b) of raw milk of poor quality. How many bacteria are visible? This field shows but a small portion of the 1/100 c.c. of milk which was spread on the slide by a special pipette (Fig. 97) covering a space of two sq. cm. How can we determine the number in the whole 1/100 c.c.? In one c.c.?

(3) Plate or count method of examining milk. Dilutions (1/10, 1/100, 1/1000) of a sample of milk were used

to make these three plates (Fig. 107). Are the relative numbers on these plates what you would expect? Why are the colonies so small in the 1/10 dilution, with a gradually increasing proportion of larger ones as the dilutions grow weaker?

2. Pasteurization

(1) To demonstrate the bacterial reduction in pasteurized milk.

a. Shake well the sample of raw milk provided, and divide it into two parts, pouring half into each

Fig. 107.—Three different dilutions of the same sample of milk. See Exercise 1, (3). (Ricker.)

of two sterile test tubes. Pasteurize one tube 145° F. or 62.8° C. for 30 minutes. (The less effective flash method, 176° F. or 80° C., may be substituted if the length of the class period makes the other method impossible.) Cool quickly by placing in the ice bath provided. The non-pasteurized tube serves as a control.

b. Make 1/100 dilutions of each of these two tubes.

c. With these dilutions make agar plates (See p. 181) for comparison of the live organisms present in the milk before and after pasteurization. Use one c.c. of each dilution.

d. After incubation (37° C. for two or three days), count and record on the blackboard the colonies present on each plate.

e. Average the results obtained by the class for the raw and the pasteurized sample.

f. What per cent. reduction was obtained by pasteurization?

g. How does this accord with the claims made for pasteurization?

(2) From the references at the end of this chapter, find a method of pasteurization which does not require a thermometer. Check the process with a thermometer to see if the milk is kept at a recommended temperature for a sufficient period.

3. Milk production

Consult the same references, and find the latest recommendations or conclusions regarding (1) cleaning milking machines, (2) sterilizing milk cans, (3) cleaning cows before milking, (4) pasteurizing in bottles or in bulk, (5) capping milk bottles, (6) vitamines in pasteurized milk, and (7) dairy score cards.

4. Diseases carried by milk. (Those shown under Water are not repeated.)

(1) Diphtheria. Note the unequal way these organisms stain with methylene blue (Fig. 64, II). Why would a doctor be justified in refusing a dairyman permission to continue at his work solely on the basis of a throat swab showing such organisms?

(2) Human tuberculosis. Tuberculosis organisms (as earlier described) stain with difficulty (waxy envelope); once stained, they hold the stain even when bleached in strong acid. They are therefore called acid-fast. Few, if any other, acid-fast organisms are found in sputum; finding such organisms is therefore usually considered conclusive. Note, also, the characteristic, finely-granular appearance of these rods (Fig. 21, II and Fig. 87).

(3) Bovine tuberculosis. Compare these (Fig. 21, II) in size and staining characters with the human type. Can the type of an infection—human or bovine—be determined by microscopic methods?

(4) Septic sore throat. Find in the references listed at the end of the chapter, one epidemic due to milk-borne streptococci.

5. Milk products

(1) Commercial sour milks

a. Make a smear * of one of the commercial sour milks (Figs. 17 and 18, II) provided to determine the general types of organisms present: molds, yeasts, cocci and rod-shaped bacteria.

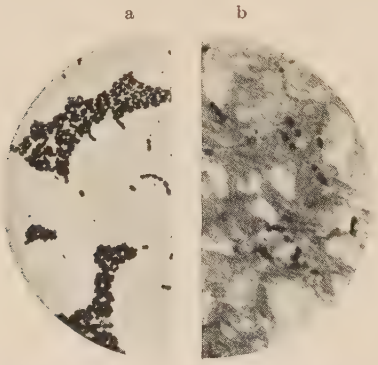

Fig. 108 a.—Bacteria (*Streptococcus lactis*) important in cheese making. The right half, b, shows the organisms developing in the curd stage of American or Cheddar cheese. (Russell and Hastings: Outlines of Dairy Bacteriology, Russell.)

b. Compare with smears of the other milks made by various students.

c. Taste the various milks. (Used glasses must be immediately carried to the sink, not left on the table.) What types of organisms are present in the milk you prefer?

d. Inoculate one of the pasteurized milk tubes with the preferred milk culture, recording carefully the types of organisms present in the milk used for inoculation.

e. At the next period determine the purity of your transfer.

f. Make recommendations for home production of such milks.

(2) Cheeses and butter

a. Examine the cheese slides provided: cream, Limburger, Cheddar, Roquefort, Swiss, etc., and note

* Use the alcohol and xylol method of fixation advised earlier for cheese (p. 64).

differences—kinds of molds and bacteria, and relative abundance. Are the differences marked enough for you to recognize a slide when the label is not visible? (See also Fig. 108.)

b. Find in the references provided two cheeses for which the essential organisms have been determined.

c. For what dairy products is pasteurization recommended?

d. What organisms are considered desirable in butter making?

e. What organisms are sold commercially as starters for butter?

f. Name three organisms responsible for spoilage of cheese or butter.

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REVIEW AND STUDY QUESTIONS

1. What are the avoidable sources of bacteria in milk?
2. How may the bacterial content of milk be kept at a minimum?
3. How may the bacterial content of milk be determined?
4. Why is refrigeration emphasized for milk and not for water?
5. Refute or balance the arguments against pasteurization.
6. What human diseases have been traced to milk? Which came from other human beings? Which of these originated in the cow?
7. Why are we justified in condemning milk on high bacterial counts only?
8. Formulate directions for caring for milk in the home.
9. Give simple directions for home pasteurization of milk.
10. What conditions minimize the dangers from pathogenic organisms in butter? In sour milk drinks or products?
11. What are "starters" in butter and cheese-making?
12. What phases of milk (and milk products) are of most interest to investigators today?

CHAPTER X

SOILS AND THE NITROGEN CYCLE

LECTURE AND READING OUTLINE

A. Soil relations which are not bacterial

(A) Water. Excess or lack met

a. Directly: by drainage or irrigation

b. Indirectly: (a) forest relations; effect of leaf transpiration and air moisture on frequency of rainfall, and effect of roots in soil on its water-holding power; (b) changes in physical condition; *e. g.*, lime, with its resulting coagulating action on organic matter, improves texture of both clay and sandy soils

Fig. 109.—Variations in mineral supply and in seedling growth. The control bean seedlings have iron (Fe), phosphorus (P), nitrates (N), potassium (K), calcium and magnesium. Poorer growth follows when any one is omitted; note the lack of chlorophyll if iron (Fe) is not supplied. (Richards.)

(B) Minerals

a. Uses

(a) Building material or cell substances

(b) Stimulants or controlling factors in growth (Fig. 109); normal growth a bal-

ance of stimulating and inhibiting factors, *e.g.,* calcium stimulates and potassium inhibits activity of certain cells

b. Selection of mineral
   (a) Related to original soil content; importance of securing analysis before fertilizing additions are made
   (b) Related to prospective crop; *e.g.,* nitrates, useless expenditure for leguminous crop which bears nitrate-forming nodules; must suit crop's special demand, *e.g.,* phosphate
   (c) Related to other soil additions at time: manure, turned-in crops (cow-peas)

c. Available nitrogen the main mineral problem
   (a) Nitrogen as basal element of all proteins and therefore all living cells
   (b) Nitrogen not usable by green plants as nitrogen gas, but only in such combinations as nitrates
   (c) Nitrogen is lost to the soil in the decay of nitrogenous matter (manure, rotting plants, decomposing animals); during the disintegration of such substances a small amount of nitrogen is transitorily available in absorbable organic form (amino-acids), and a small amount as salts (nitrates), but most of the N passes into the air as nitrogen gas (N), or ammonia (NH_3)

d. Replacing the soil nitrogen
   (a) Nitrate salts (KNO_3, $NaNO_3$, etc.), commercial fertilizers or "by-products" of commercial processes
   (b) Manure or similar animal wastes
      1. Forms
         (1) Ordinary farm manure
         (2) Guano; bird deposits (Peru)
         (3) Refuse from fisheries, packing houses, etc.

(c) Green plant additions
1. Turned-in crops, stubble, roots, leaf decay
2. Wood ashes

(d) Fixation of atmospheric nitrogen
1. By flame (hydrogen gas)
2. Electricity
(1) In nature: electric discharges, as in thunder showers
(2) With machines: electric discharge through confined air. High cost of power renders impracticable in many places; possible where water power available (Norway, numerous waterfalls)
3. Ultra-violet light fixes nitrogen, which is returned to earth by rain and snow. Estimated at 100,000,000 tons a year, yet almost negligible, in view of great area of earth's surface
4. By combination with dextrose and cellulose (rotting wood)

(e) Bacterial processes which supply usable nitrogen to soil; improved growth attributed to turned-in crops or stubble, to manure, etc., is dependent upon bacteria, which make such materials more available. See B following

B. Bacterial relations

(A) Decomposition or denitrification; mainly by bacteria, though molds are helpful, sometimes essential, in beginning stages of decay

a. Types or stages

(a) Decaying of plant and animal tissues to nitrogen or to ammonia

(b) Such partial or intermediate stages as urea to ammonium carbonate

(c) Term denitrification more often limited to the last breaking-down stages, nitrates to nitrogen (N) or ammonia (NH_3). See (d) following

b. Such organisms numerous and wide-spread, forming at least 75 per cent. of several large series of organisms investigated; to be expected as

(a) Utilization of protein substances a common cell function

(b) Decay occurs in widely varying conditions; common organisms are active agents: *Bacillus subtilis, Proteus vulgaris,* etc.

c. Conditions affecting nitrogenous decomposition

(a) Temperature; slow rate of decomposition at cold latitudes and altitudes, even when decay organisms are present; wide range in optimum temperatures, some approximating freezing

(b) Activity related to the presence of oxygen: mainly aërobic; some anaërobic forms grow with associated aërobes which use up free oxygen

(c) Dependent upon the presence of nitrogenous material; number in rich loam may reach millions per gram

d. The term denitrification quite commonly limited to changing nitrates (KNO_3) to nitrogen or ammonia, the last phase of the decomposition of nitrogenous matter. The nitrates are reduced, oxygen taken out, and so changed to nitrites, to ammonia, and to nitrogen. Since nitrates are the most available forms of nitrogen for green plants, such denitrification lessens the supply, though this loss may be replaced by the upbuilding action of other bacteria. (See (B) and (C) following)

e. "Leaching": term applied to wastage of utilizable nitrogenous substances, as when manure is left too long exposed to weather, when the desirable nitrates are lost, being changed to non-utilizable N or NH_3, or washed or drained away by rain, etc. In other words, denitrification may

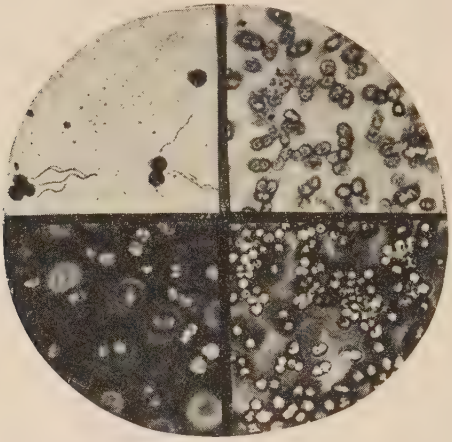

FIG. 110.—*Azotobacter agilis*, a nitrogen-fixing bacterium with a surprisingly varied cycle. Four of the cycle stages are shown above. (Jones: Bulletin Ontario Agricultural College.)

be carried on too rapidly to allow the complete absorption of utilizable stages by crops

(B) Nitrogen-fixation (by molds, also): process utilizes nitrogen, making it into salts (nitrates), combinations with calcium ($CaNO_3$), potassium (KNO_3), etc.

a. Soil bacteria

(a) Mainly aërobic

(b) Considerable resistance to drying, etc.

(c) Most common species, *Azotobacter*

(d) Nitrates deposited in soil, often 15 to 40 pounds per acre

(e) Colorado deposits used as fertilizer have been attributed to this process

b. Nodule or root bacteria
   (a) Plants bearing nodules common in bean family (Leguminosæ): clover, beans, peas, vetch, alfalfa, berseem
   (b) Until 1866 these nodules were considered pathological developments
   (c) Really a symbiosis between bacteria and plants
      1. Bacteria gain water and food, while invaded plants gain necessary nitrates
      2. Benefits even crop of present season as proved by comparing growth of peas, clover, beans, etc., with and without nodules present, and by comparing growth (average weight per plant) of timothy or other plants mixed with nodule plants
   (d) Commonest species, *Rhizobium radicicolum;* many varieties known; more than one may be found living on one plant
   (e) Species of bacteria adapted to hosts; *e.g.,* one species or variety better adapted to white clover than to red; partly a question of resistance of root-hairs of host
   (f) Nitrate formation
      1. Increased by certain organic acid salts
      2. Total nitrates formed often high, 100–200 pounds per acre
   (g) Rotation of crops in general farm practice provides one nitrate-producing crop every three to five years

(C) Nitrification: the utilization of ammonia by bacteria, which build it up into nitrates
   a. Occurrence suggested by Pasteur
   b. Experimental proof obtained by French investigators, 1877; later two organisms utilizing ammonia isolated (Winogradsky): *Nitrosomonas* and *Nitrosococcus*

c. Chemical changes, ammonia (or ammonia salts) to nitrates, involve two different species: one oxidizes ammonia to nitrites, the other, nitrites to nitrates

(a) Ammonia to nitrites produced by organisms having a definite cycle in development (Fig. 116). The different species are local in distribution and inhibited by the presence of organic matter; CO_2 may be utilized

(b) Nitrites to nitrates (*Nitrobacter*) inhibited by the presence of ammonia, less affected by organic matter than the nitrite formers

(D) Bacteria and nitrogen cycle

a. Complete biological cycle (Fig. 117) showing interdependence of green plants and bacteria

b. Crosscut in cycle—urea to ammonia by animals —does not break cycle

c. Nitrate to nitrogen and ammonia may cause reversal of nitrates built up by bacteria from nitrogen and ammonia; this merely delays and does not break the cycle

d. Comparison with other cycles, such as oxygen, sulphur or carbon cycles, shows

(a) That, unlike the nitrogen cycle, they are not so limited to biological relationships or processes; *e.g.*, O to CO_2 in combustion is not preëminently a biological process; and

(b) In them green plants and micro-organisms are not definitely alternating agents, as in the nitrogen cycle

C. Other methods of improving soils

(A) Allowing ground to lie fallow; may improve a given soil by changes in

a. Physical condition; open to frost and sun

b. Minerals may be increased by bacterial activity; molds, bacteria, insect pests decreased by leaving soil open to winter cold and summer heat; aëration may lessen molds, etc. Effects not always

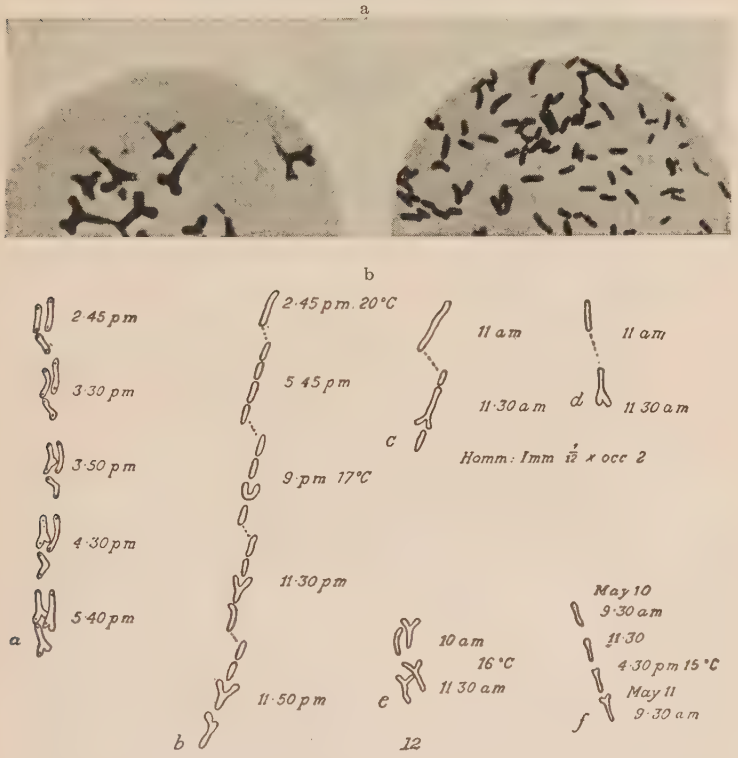

Fig. 111 a.—The upper illustration shows two forms of *Rhizobium radicicolum:* left, branching forms from clover; right, rod forms from fenugrec (a clover-like plant). (Reduced from Moore, Year Book, U. S. Department Agriculture.)

b. The drawings below show stages in the formation of branching forms with time intervals indicated. (After Maria Dawson.)

beneficial; weed crop may exhaust given soil as much as a salable crop which makes possible the purchase of replacing fertilizers

(B) Heating soils

a. Effect on mold and insect pests beneficial

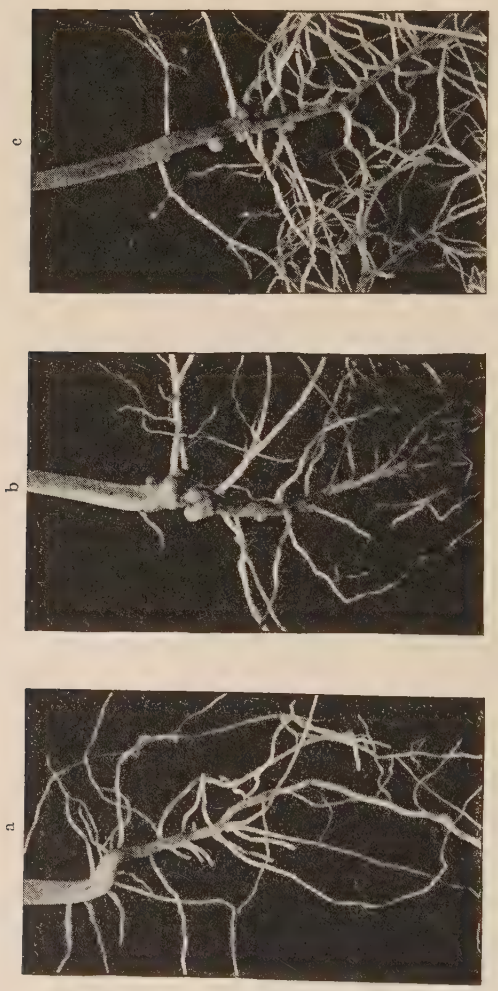

FIG. 112.—(a, b, c) These cowpeas were grown in various mineral solutions. Left, normal or control plant; center, with chlorides; right, with calcium. Notice how much chlorides and calcium compounds aid in nodule formation. (Wilson, Cornell Agricultural Experiment Station.)

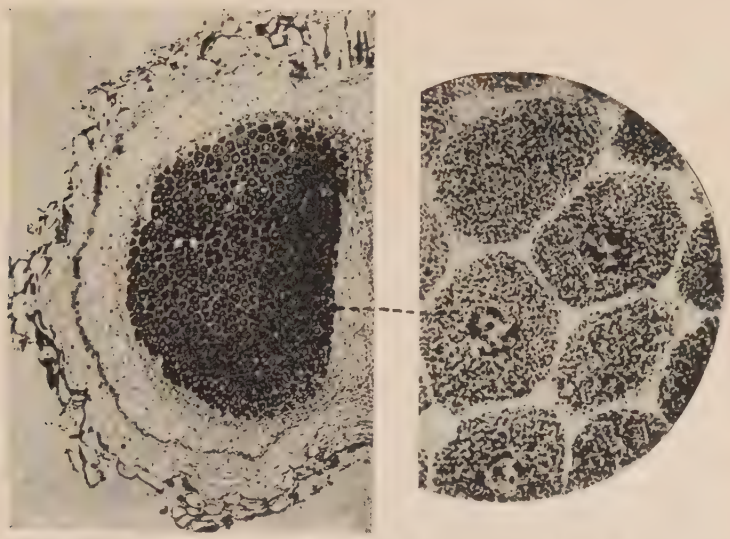

FIG. 113.—Sections through nitrate-nodules (lupine): the one on the right is more highly magnified and shows more distinctly the bacterial masses within the root cells. (Reduced from Moore, U. S. Dept. Agriculture.)

FIG. 114.—The importance of nitrates is illustrated by the two halves of a field showing differences in the early growth of corn (1921). Yield left half, with 1000 pounds of ammonium nitrate per acre, 26.8 bu. per acre; right, no fertilizer, 8 bu. per acre. (Allison, Braham and McMurtrey, U. S. Dept. Agriculture.)

b. Effects on micro-organisms various; theory that injurious organisms are killed off in larger ratio than helpful ones accepted by many

FIG. 115.—Subsequent (as well as current) crops benefit by the action of plants which carry nitrate-forming bacteria, as shown by the above illustrations, showing the relative heights of oats growing on adjacent plots of ground. The oats crop following clover was richer in grain yield as well as straw. (After Gustafson, N. Y. State College of Agriculture.)

FIG. 116.—A much-magnified surface colony (agar plate) of soil bacteria (*Nitrosomonas*) which utilize ammonia and manufacture nitrites. Later, *Nitrobacter* organisms change the nitrites to nitrates. (Gibbs, Soil Science.)

c. Raises soluble organic acid and salt content temporarily; increases food possibilities in early stages, and gives plant a better start; may give more favorable proportions of a given soil mineral, stimulating germination and initial growth

DEMONSTRATION AND LABORATORY WORK

1. Using the reference list at the end of this chapter, find how the media used for growing soil organisms differ from the media used in this course.
2. Soil counts are usually made by determining the number of colonies developing from a given unit of soil. Find repre-

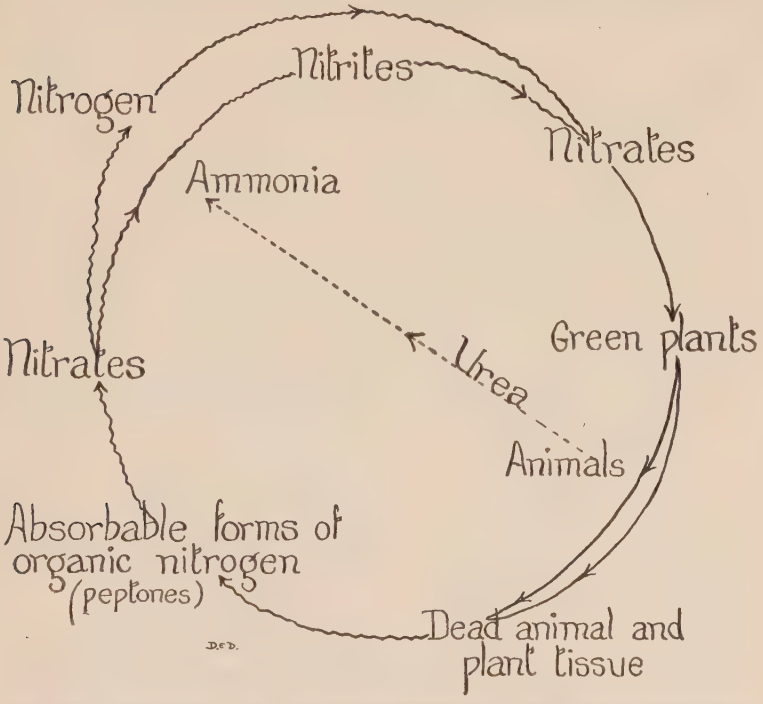

FIG. 117.—The nitrogen cycle. Compare with that given in any reference text, and fill in with the appropriate names of the associated bacteria.

sentative counts in the references provided. Give one explanation of the higher count in the surface soil.

3. Soil organisms, *Azotobacter agilis* (Fig. 110); these, like the nodule bacteria, utilize the air nitrogen to make nitrates.
4. *Rhizobium radicicolum*. Compare the diagram with the illustration Fig. 111). Which shows a greater range in cell shapes?

5. Pea roots (Fig. 112), showing nodules, filled with bacteria, *Rhizobium* (formerly *Bacterium*) *radicicolum*. One of these nodules was sectioned to make the following slide (Fig. 113).

6. In this section of pea roots, find granular-looking cells, containing *Rhizobium radicicolum;* the normal cells are much smaller and lack the heavy granular appearance due to these bacteria.

7. Photograph (Fig. 114) showing crops grown in the same soil, with and without nitrates. Are nitrates necessary for normal development?

8. Photograph (Fig. 115) shows a crop grown with and without nitrate-forming bacteria. This shows that leguminous plants, such as peas and beans, can, with the aid of bacteria, supply their own nitrates.

9. Organisms which utilize ammonia (NH_3) and so help store nitrates in the soil (Fig. 116). What is the stage following the action of *Nitrosomonas?*

10. Redraw this nitrogen cycle, adding the names of bacteria in the sections where they serve to complete the cycle (Fig. 117).

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REVIEW AND STUDY QUESTIONS

1. How is agriculture affected by denitrifying organisms?
2. What are the two main types of nitrogen-fixing bacteria? How do they differ?
3. How is ammonia made available as green plant food?
4. Draw a diagram illustrating the nitrogen cycle, and show how each phase of it affects our welfare.
5. Since nitrates are constantly formed as plants decay, why do we emphasize the need for the upbuilding of nitrates from nitrogen and ammonia?
6. What practical contributions to soil bacteriology have been made in the last ten years? (See Current Economic Bacteriology.)

CHAPTER XI

OTHER ECONOMIC PHASES OF BACTERIOLOGY

LECTURE AND READING OUTLINE

A. Removal or inhibition of micro-organisms

(A) Heat

a. Canning

(a) Early dispute over "spontaneous generation" centered around processes similar to canning

(b) Discontinuous method useful when resistant spores are present; one-day intervals allow germination, and vegetative stages are killed in subsequent heating

b. Preserving, longer heat exposure; better keeping qualities of product related to

(a) Lowered water content (prolonged boiling) and higher sugar content; germinating organisms plasmolyzed

(b) Absence of oxygen due to long boiling; spoilage usually limited to (better aerated) surface of jar

(c) Formation of formaldehyde (?) by prolonged heating

c. Soil (See also Soils and the Nitrogen Cycle)

(a) Rothamstead theory: heating removes undesirable micro-organisms which affect desirable soil organisms

(b) Relation between heated soils and per cent. of soluble salts and organic acids may account for much of improvement in crops (see p. 198)

(B) Salt additions, as brines and pickles
  a. Salted fish, meats, etc.
    (a) Occasional spoilage due to salt-resistant organisms. Recent spoilage of salt cod due to red protozoa and bacteria in salt from tropics; very resistant, multiplying in saturate salt solution
    (b) Bouillon cubes—50–74 per cent. table salt explains "keeping" qualities

(C) Removal of water
  a. Water content too low to favor growth, surfaces of dried meats, etc.
  b. Additional precautions necessary
    (a) Sterile, non-absorbent containers, as in dehydrated vegetables, dried milk, eggs, etc.
    (b) Preservatives sometimes allowed: sulphur gas in dried apples (color condition improved)
  c. Spoilage often less than if left in natural state, *e.g.*, dried eggs and whole eggs

(D) Low temperatures
  a. Range in present applications: home icebox to refrigerating plants and cars; fruits to whole animals; foods to fur coats
  b. Advantages
    (a) Control of micro-organisms
    (b) Conservation and distribution of seasonal and temporary products: eggs, fruits, etc.
    (c) Meats and fish
      1. Results of chemical tests (fish) show no deterioration in two years
      2. Effective against parasites (trichina)
      3. Advantage gained through U. S. Inspection required on interstate exchanges, the long distances insuring cold storage for major part of meat supply.

c. Attending evils
(a) Deterioration during storage
1. Cell enzymes in original substances themselves (canned fruit) not eliminated by storage
2. Bacteria multiply in substances
(1) Which cannot be kept at approximately freezing or below, *e.g.*, eggs
(2) If initial bacterial content high, as in intestines of chickens, when odors and bacteria themselves may spread into flesh
(b) Repeated thawings, especially in water, favor bacterial growth
1. Formerly done to conceal storage character; goods should be kept chilled until sold (or used)
2. Illegal, in wide-awake communities

(E) Chemical additions
a. Allowed in few cases
(a) Dried apples (sulphur gas)
(b) Substances left exposed to contamination: jams in bulk, bottled catsups (benzoate of soda)
b. Objections to chemicals
(a) Possible effect on consumer by long-continued use, even in small amounts
(b) May make food less digestible (fix or harden tissue)
(c) Sometimes used to mask poor quality (changes in color and texture)
(d) Labels do not protect ignorant consumers

B. Growth of desired organisms. Bread, milk and soils (treated elsewhere) belong here also
(A) Soured and fermented foods, with or without brine additions

a. Formation of acids (lactic prominent) limit growth of spoilage organisms
b. Substances such as sourkrout, some catsups, corn
c. Silo
   (a) Lactic acid prominent, limiting further change in silage
   (b) Heat due to bacterial activity and to oxidation by live green cells (silage) also important controlling factor

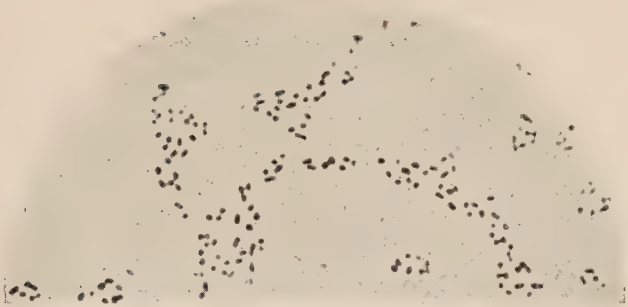

FIG. 118.—Vinegar bacteria. (Wyant, Michigan State College of Agriculture.)

(B) Alcoholic fermentation: sugars, fruit juices, starchy substances to alcohol
   a. Yeasts prominent; types vary in amount of alcohol formed before growth inhibited; may reach 16 per cent.; higher percentages obtained by distillation of alcoholic substances, or addition of alcohol
   b. Bacteria may be important in producing flavor
   c. Spoilage due to molds, undesirable types of yeasts
   d. Additions (hops, etc.) to give flavor, inhibit undesirable organisms

(C) Acetic fermentation: vinegar making
   a. Alcohol oxidized to acetic acid (see p. 55)
   b. Action depends on alcohol present (may be inhibiting amount); ceases with high acetic acid content (14 per cent.)
   c. Spoilage due to organisms using acetic acid: bacteria, "vinegar eels," etc.

(D) Commercial processes where bacterial changes are as yet poorly or incompletely controlled

a. Tanning of leather

(a) Bacterial action aims at balance between removal of much of fat or oil, and "rotting" of leather

(b) Present chemical processes claim to be practically perfect, eliminating early crude

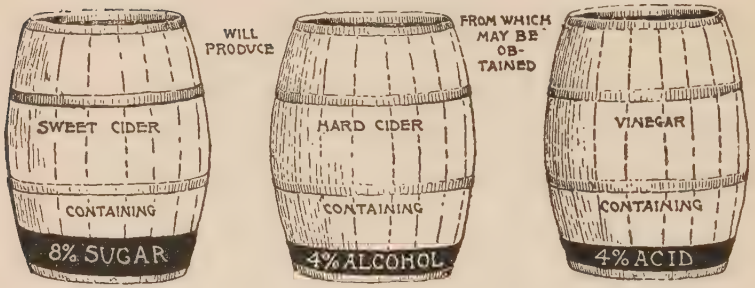

FIG. 119.—Yeasts often make the first change given here; there are, however, yeasts which can complete the whole process without aid from bacteria. (After Rice N. Y. State College of Agriculture.)

bacterial methods (additions, such as manure)

b. Textiles

(a) Separation of fibers (leaves and stems) in the textile industry

(b) Bacterial cultures, known organisms, may be used to bleach fibers (cotton, linen); action less destructive than bleaching chemicals

(E) Processes depending markedly on changes due to enzymes in cells of tissues concerned (and probably less upon bacteria and their enzymes)

a. Methods—adapted to securing optimum conditions, such as

(a) Temperatures, *e.g.*, "sweating"

(b) Oxygen supply

b. Plants, etc., used

(a) Indigo

1. Sources—plants (indican); coal tar supplanting such sources

2. Cell enzymes important in plant production
   (1) Shown by factors affecting product (time of day picked, etc.)

(b) Tobacco
   1. Method of drying, ripening
   2. Changes produced
      (1) Loss in nicotine
      (2) In aroma and color; slow drying increases changes in cell substances and reduces the rank odor when smoked

(c) Tea
   1. Quality depends on relation of oils, unfermented tannin, and "body," as cell enzymes change contained substances in tissues of tea leaves
   2. Bacteria really necessary?
   3. Important considerations
      (1) Rate of drying: (too slow, molds develop; too rapid, enzymes cannot complete changes)
      (2) Heat; related to drying rate
      (3) Antiseptics to control molds when drying is slow

(d) Cocoa
   1. Partial fermentation
      (1) Chemical effects: taste; color
      (2) Physical gains: loosens seed coat and pulp from seed

(e) Coffee (changes much as in cocoa)

(f) Meats
   1. Cell enzymes cause *rigor mortis;* later softening of cell membranes (tender meat) also due to enzyme action
   2. "High" or "gamy" flavors due to bacterial changes

(F) Cellulose. Enzymes decomposing cellulose important in
   a. Plant decay
   b. Digestion of foods by animals
      (a) Cellulose of plant cell walls indigestible for man
      (b) Food in cells liberated by
         1. Breaking or destroying cell walls
            (1) Cooking
            (2) Crushing

(3) Chemical action on cellulose, herbivorous animals (intestinal juices). Cellulose itself of food value

2. Bacteria digesting cellulose, helpful, herbivorous animals
   (1) Common in soil
   (2) Alimentary canal of such animals

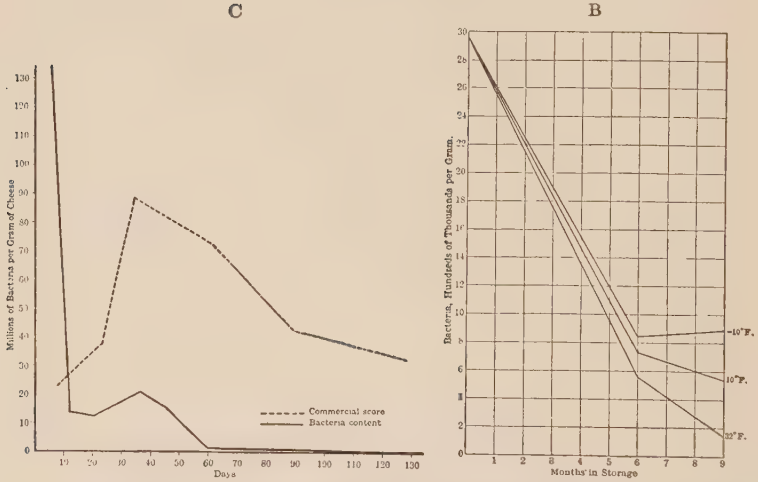

FIG. 120.—Bacterial decrease with age or storage. C, cheese of the Cheddar type (after Harding and Prucha, 1903); B, butter, stored at different temperatures. (After Mohler, Washburn and Rogers.)

(G) Sewage. Disposal mainly a problem of mineralizing such organic matter as human wastes
   a. Types of bacterial action
      (a) Aërobic organisms: oxidizing or nitrifying action
      (b) Anaërobic organisms: putrefying action
   b. End products
      (a) Vary with organisms and the degree to which they act upon the organic wastes present in sewage

(b Prominent or common end products: carbon dioxide, hydrogen, marsh gas, mineral salts (nitrates) and ammonia

c. Mechanical devices to aid desired type of action

(a) Oxygen supply increased by

1. Porous (air-holding) beds of coke, stones, etc.
2. Spraying the sewage into the air
3. Forcing air (oxygen) into tanks of sewage

(b) Anaërobic (putrefying) action insured by

1. Covered tanks
2. Depth of liquid itself (tanks uncovered); affinity of organic matter for oxygen leaves no free oxygen and so favors anaërobic bacteria

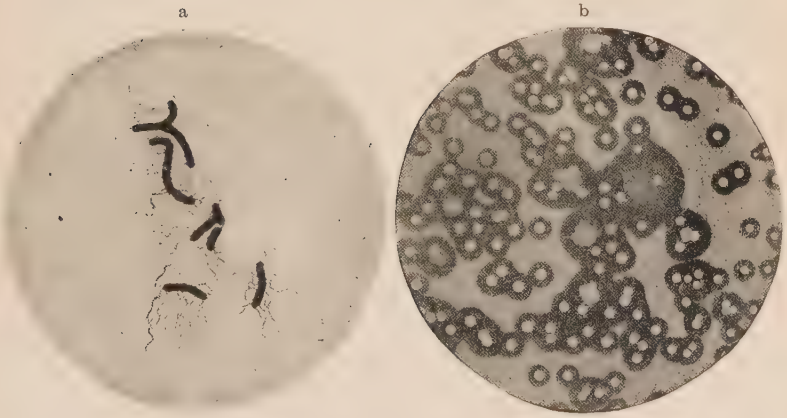

FIG. 121.—(a, b) A cellulose-digesting bacterium (*Cellulomonas cytaseus*) is shown on the left. In the cellulose-agar plate on the right the digestion of cellulose is shown by the clear area surrounding each colony. (McBeth and Scales, U. S. Dept. Agriculture.)

DEMONSTRATION AND LABORATORY WORK

1. Organisms (Fig. 118) from "mother of vinegar." From the references listed at the end of this chapter, find two vinegar organisms which differ distinctly from this one.

2. Find two ways in which bacteria may be used in textile industries.

3. Name two organisms prominent in silage.

4. Name one organism for each of the two types of bacterial action occurring in sewage disposal.

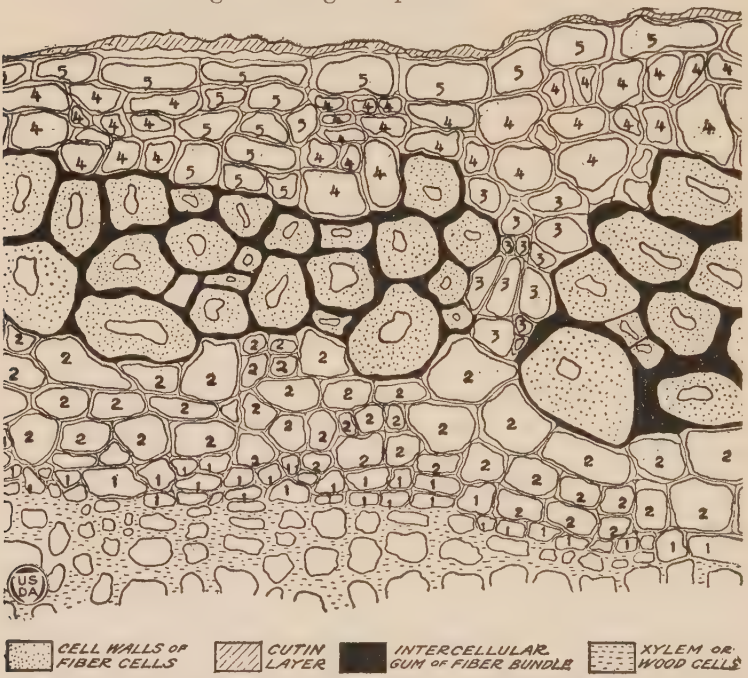

FIG. 122.—A diagram of a flax stem, showing the order in which the cells are rotted or destroyed by bacteria: 1, cambium; 2, 3 and 4, the soft tissues between and around the veins; 5, the epidermis. This leaves the fiber cells, shown above, surrounded by the gum (black) which practically sheathes the thick-walled fiber cells. (Davis: Flax Stem Anatomy in Relation to Retting. U. S. Dept. Agriculture.)

5. Diagram two different methods of supplying oxygen to aid in the mineralization of sewage.

6. List under (1) helpful and (2) undesirable organisms at least six important food organisms.

7. Plant diseases. Most important economically are the diseases that affect our staple crops. Many of these are due to fungi;

FIG. 123.—Above, test showing incompletely retted flax; note large pieces of epidermis at e. Retting is about complete in the lower illustration. (Davis: Flax Stem Anatomy in Relation to Retting. U. S. Dept. Agriculture.)

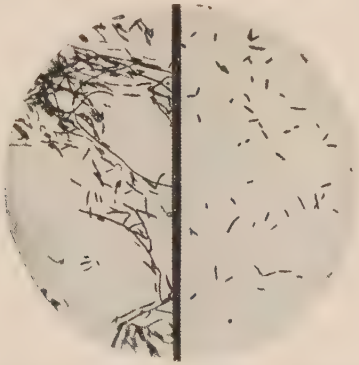

FIG. 124.—Two different stages (variations in 24-hour cultures) of bacteria (*Phytomonas pisi*) parasitic on peas. (Sackett: Colorado Ag. Exper. Station.)

bacteria, however, are responsible for a large share of the yearly losses.

Look through the selected pamphlets on bacterial diseases which illustrate the work being done by the U. S. Government, the state experiment stations and agricultural colleges.

Fig. 126

Fig. 125.—Wilt of cucumber and squash due to parasitic bacteria, *Erwinia tracheiphilus*. (Jones: Some Bacterial Diseases of Vegetables Found in Ontario.)

Fig. 126.—Cucumber wilt is directly due to lack of water as the water tubes become clogged with bacteria (*Erwinia tracheiphila*). The viscid character of the bacterial mass causes the threads formed as the finger is drawn away from the cut surface. (Smith: Bacteria in Relation to Plant Diseases, Carnegie Institution of Washington.)

What important crop apparently has no disease due to microorganisms? How long a list of *bacterial* diseases can you make? Name one in which control is maintained by spraying or otherwise affecting the parasite; one, in which successful crops depend upon resistant types of the plant, such as rust-resistant beans (see p. 70, also Frontispiece).

8. Animal diseases. These cause great losses to our country

FIG. 127.—These three photographs show spoilage due to very different types of causes; in the first the changes are due to a fungus (*Anthracnose*), in the second to sun scald, and in the lowest one to a bacterium (*Phytomonas phaseoli*). (Barrus, Burkholder and Hawley, N. Y. State College of Agriculture.)

every year. Their importance may be judged by the (marked) publications listed at the end of the chapter. Can you find one disease controlled by vaccination (as in man for typhoid

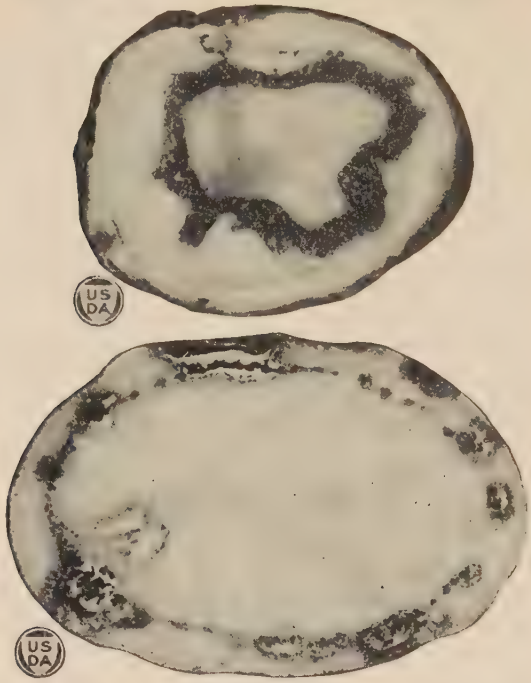

FIG. 128.—Another illustration of the superficial resemblance in injuries due to different factors. On the bottom, brown rot or southern bacterial wilt of potatoes; and on the top, black-heart, due to asphyxiation, occurring when the temperature is too high or the oxygen supply inadequate. (Shapovalov and Link, U. S. Dept. Agriculture.)

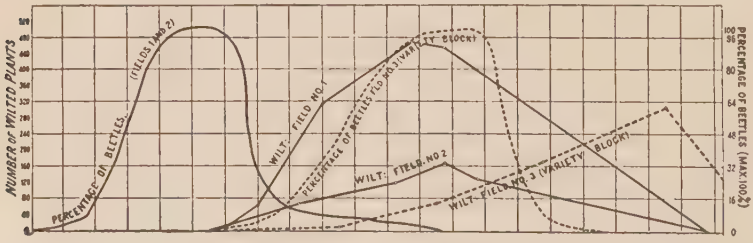
NUMBER OF WILTED PLANTS
PERCENTAGE OF BEETLES (MAX. 100%)
PERCENTAGE OF BEETLES
(FIELDS 1 AND 2)
WILT: FIELD NO. 1
PERCENTAGE OF BEETLES FIELD NO. 3 (VARIETY BLOCK)
WILT: FIELD NO. 2
WILT: FIELD NO. 3 (VARIETY BLOCK)

FIG. 129.—The relation of insects in bacterial wilt of cucumbers is shown by the increase in wilt in these three fields following the maximum records for the striped cucumber beetle (See page 70). (Rand and Enlows, U. S. Dept. Agriculture.)

or smallpox)? Are resistant-varieties (Algerian sheep to anthrax) advised for any species other than sheep?

9. Select from the Meat Inspection of the U. S. Department of Agriculture, 1914, five important regulations relating to animal diseases transferable to man.

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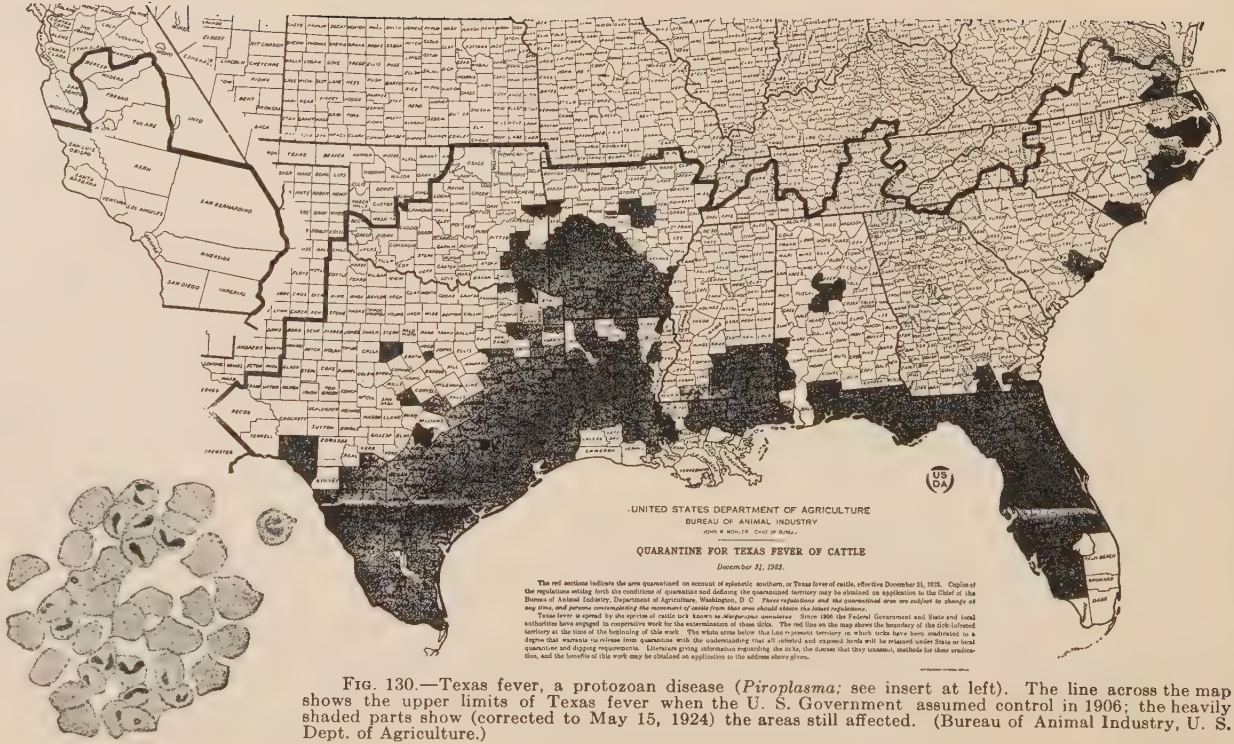
UNITED STATES DEPARTMENT OF AGRICULTURE
BUREAU OF ANIMAL INDUSTRY
JOHN R. MOHLER, CHIEF OF BUREAU
QUARANTINE FOR TEXAS FEVER OF CATTLE
December 31, 1923.
The red sections indicate the area quarantined on account of splenetic southern, or Texas fever of cattle, effective December 31, 1923. Copies of the regulations setting forth the conditions of quarantine and defining the quarantined territory may be obtained on application to the Chief of the Bureau of Animal Industry, Department of Agriculture, Washington, D. C. These regulations and the quarantined areas are subject to changes at any time, and persons contemplating the movement of cattle from that area should obtain the latest regulations.
Texas fever is spread by the species of cattle tick known as Margaropus annulatus. Since 1906 the Federal Government and State and local authorities have engaged in cooperative work for the extermination of these ticks. The red line on the map shows the boundary of the tick-infested territory at the time of the beginning of this work. The white area below that line represents territory in which ticks have been eradicated to a degree that warrants its release from quarantine with the understanding that all infected and exposed herds will be released under State or local quarantine and dipping requirements. Literature giving information regarding the ticks, the disease that they transmit, methods for their eradication, and the benefits of this work may be obtained on application to the address above given.

FIG. 130.—Texas fever, a protozoan disease (*Piroplasma;* see insert at left). The line across the map shows the upper limits of Texas fever when the U. S. Government assumed control in 1906; the heavily shaded parts show (corrected to May 15, 1924) the areas still affected. (Bureau of Animal Industry, U. S. Dept. of Agriculture.)

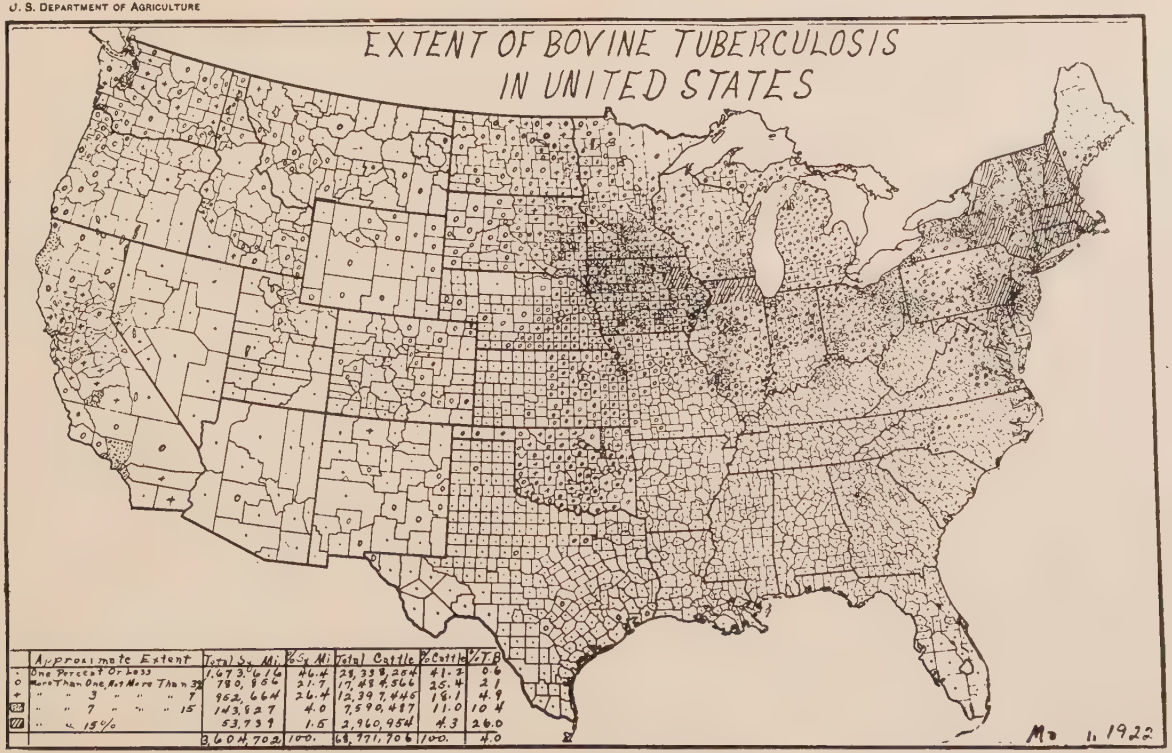

FIG. 131.—Bovine tuberculosis in the United States. The average for the whole United States (1923) was estimated at less than 4 per cent. What areas need most attention? (Bureau of Animal Industry, U. S. Dept. of Agriculture.)

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REVIEW AND STUDY QUESTIONS

1. List the home and commercial operations and processes which are designed to destroy or inhibit micro-organisms.

2. List, similarly, those in which one or more kinds of desired organisms are encouraged to develop.

3. Name five important bacterial diseases of plants; five fungous diseases.

4. Name five important bacterial diseases of domestic animals; three due to animal parasites.

5. Prepare two lists of helpful and harmful organisms with the related process, food, industry, etc. (See Current Economic Bacteriology as well as all the standard texts.) Do any organisms appear in both lists?

CHAPTER XII

MICRO-ORGANISMS AND HUMAN DISEASE

(This chapter forms a brief introduction to Vol II, How We Resist Disease)

LECTURE AND READING OUTLINE

A. Cause of human disease
  (A) Diseases which are not transferable; illustrated by such types as
    a. Nutritional (scurvy, pellagra, rickets)
    b. Functional (kidney, thyroid and pancreas disturbances)
    c. Due to chemical poisons (lead, arsenic, phosphorus in various industries)
  (B) Communicable diseases, due to micro-organisms
    a. Animal parasites
      (a) Many-celled animals
        1. Mites and ticks; types:
          (1) The itch mite which burrows in skin, causing itch
          (2) Sucking ticks, on surface of body, as in relapsing fever or Texas fever
        2. Worms; commonly intestinal infections
          (1) Tapeworms of cow or pig, often transferred by infected flesh eaten by man
          (2) Round worms: trichina, transferred to man in uncooked flesh (pig); hookworm, enters through unbroken skin, passing to lymphatics, heart, lungs, throat and finally to intestines

(b) Protozoa or single-celled animals

1. Types varied: from simple ameba (dysentery, Fig. 5, II) to complex cycle types (malaria organisms, Figs. 58, 59)
2. Alternate hosts among lower animals (mosquito in yellow fever and malaria; louse in typhus fever) more common in protozoan than in bacterial diseases (rat and flea in plague)
3. Chemical or drug treatment often directly helpful: quinine, malaria; arsenic compounds in syphilis

b. Plant parasites

(a) Many-celled plants (See molds, including yeasts, p. 56)

(b) Bacteria

1. Causal organisms in most of the communicable diseases of our latitudes
2. As yet rarely treated effectively by drugs; used helpfully in leprosy (chaulmoogra oil) and wound infections (aniline dyes)

B. How diseases are named

(A) From characteristic lesions, effects, etc.; tubercules in tuberculosis (Fig. 130, II); yellowish color of skin in yellow fever; rabid condition in rabies

(B) From locality affected: lung (*pneumon*) in pneumonia; the covering membranes (*meninges*) of brain or spinal cord in meningitis

(C) From associated organisms: actinomycosis from causal mold (*Actinomyces*), or amebic dysentery for type of dysentery due to intestinal amebæ. Really rare relationship, the organisms being oftener named for the related and earlier known disease, *e.g., Bacillus pestis,* discovered in 1894, was named for " the pest " or " the plague " known long before the 14th century

C. Disease organism transfer
  (A) Method related to location of organism causing infection
    a. External lesions; contact, more or less direct, as in diphtheria (throat), and trachoma (eye)
    b. Alimentary canal; transferred with intestinal wastes (typhoid, dysentery, cholera) by various agencies: polluted drinking water, flies, soiled hands. Commonly called "filth diseases"
    c. Blood or internal tissues, as in malaria: transferred by biting insects
  (B) Vigor of disease organisms affected by exposure to external conditions: cold, sunlight, drying
    a. Related to time exposed to air; few diseases now considered truly air-borne
    b. Lessened by possession of capsule (waxy coating of tuberculosis organisms) or covering of mucus, sputum, etc.
  (C) Often limited by "preferred method of entrance"
    a. Skin: hookworm, thinner skin areas (between the toes) the route in 90 per cent. of cases
    b. Subcutaneous tissue, poor in oxygen: tetanus, entering through wounds
    c. Respiratory tissues: influenza, pneumonia
    d. Alimentary canal: dysentery, typhoid
  (D) Lessened by normal conditions in body
    a. Skin itself
      (a) Entrance through unbroken skin, as in hookworm, really rare; breaks (cuts or other injuries) ordinarily necessary for invasion
      (b) Bacteria commonly present on skin. Infection following injury may be due to organisms on skin, from object causing injury, or later contacts with injured area (fingers, wrappings)
    b. Secretions which mechanically wash exposed surfaces (tears, eye; nasal secretions, nose); often somewhat germicidal, also

c. Unfavorable growth conditions met in the body
   (a) Fluids, such as bile in intestine or hydrochloric acid in stomach. Often overemphasized; commonly inhibitive rather than germicidal
   (b) Oxygen relations. Most tissues too little free oxygen for growth of most types of invading organisms
   (c) Germicidal character of blood. Greatly increased in disease (see E following)

D. Injurious effects produced by pathogenic organisms
   (A) Toxins
      a. Produced outside the body, below body temperatures; injury due to accumulated toxins in infected foods (botulism due to sausage, canned vegetables, Fig. 63, II)
      b. Produced by bacteria multiplying in body
         (a) Effects vary with affinity of toxin for specific body tissues (lockjaw or tetanus for nerve tissue)
         (b) Effects related to effect of toxin: dissolving tissue, paralyzing muscle cells
   (B) Formation of poisonous split-proteins
      a. From food substances in the intestine, occurring when predominant organisms are of wrong type, as in "auto-intoxication"
         (a) Food selection may prevent or minimize by giving substances less likely to be so broken down by the bacteria present in alimentary canal
         (b) Substitution of competing and less irritating organisms
            1. Metchnikoff's bulgaricus bacillus (Fig. 18, II)
            2. Acidophilus bacillus more permanent substitute (Figs. 14–19, II)
      b. From bacteria themselves, as they are disintegrated in body reactions against them (endotoxins of early bacteriologists?)

E. Reactions of the body to disease organisms
  (A) Increase in white corpuscles (normally 9,000 to 13,000 to cu. mm. of blood)
    a. Increase in total number of white corpuscles
      (a) Mainly an increase in polynuclear leucocytes (evident in abscesses)
      (b) Increase often astounding; records of over 250,000 to 500,000 per cu. mm. reported
    b. Change in proportion of different types of white corpuscles: main increase, leucocytes in most bacterial infections; lymphocyte increase more marked in tuberculosis or scarlet fever
  (B) Formation of definite anti-substances or antibodies against invading organisms
    a. Origin
      (a) Local or adjacent tissue (boils)
      (b) Lymph nodes and marrow (bone) tissue thought usually important sources
    b. Effects of such antibodies
      (a) Neutralize toxins (antitoxins)
      (b) Affect the organisms themselves (antibacterins)
        1. Destroy bacteria (lysins)
        2. Modify bacteria; their activity, resistance to white corpuscles (agglutinins, opsonins)
  (C) Immunity related to antibody production
    a. Antibodies appear early stages of disease; often present in large quantities before disease symptoms pronounced; in such cases helpful in diagnosis (typhoid, by agglutinin or Widal test)
    b. "Crisis," name given to period when total antibody productions and reactions might be expected to overbalance growth of disease organisms
    c. Persistence of antibodies after recovery gives continued immunity to specific disease organism
      (a) Factors affecting such persistence or the length of the immune period

1. The disease: for life in smallpox to but few days or none at all in "colds"
2. The severity of the attack: shorter in light than in severe attacks; shorter after vaccination than after the disease itself (smallpox)
3. Individual differences: some individuals not immune to diphtheria after first attack; explained by formation of but a small surplus of antibodies, or by their rapid elimination after recovery

F. Methods of securing or inducing resistance to disease
   (A) General body conditions
      a. Greater when body is in good physical condition: well nourished, not experiencing great fatigue, nervous strain, etc.
      b. Experiments with vitamines indicate greater susceptibility when diet is low in such essential substances
   (B) Use of vaccines to induce body to produce specific antibodies or immunity
      a. Name "vaccine" originally given by Jenner to organisms taken from cow (vacca) to inoculate into man
      b. Types of vaccines
         (a) Weakened organisms: weakened by drying (rabies); animal passage (cow for smallpox)
         (b) Killed organisms: heat, grinding or chemicals (typhoid; dysentery)
         (c) Bacterial products
            1. Toxins; *e.g.,* from diphtheria organisms
            2. Modified to allow increased dosage and thus induce greater reaction by body, *e.g.,* by addition of antitoxin in "toxin-antitoxin" now used as diphtheria vaccine

(d) Vaccines against other proteins (pollens, foods such as eggs) often used; results less marked or less permanent than in bacterial vaccines. (See Figs. 137, 138, II)

(C) Injection of blood from immune people or other immune animals (Figs. 57 to 59, II)

a. Reasons

(a) To prepare for anticipated need (child exposed to diphtheria)

(b) To help individual actually ill with related disease (pneumonia, diphtheria)

b. Substances used

(a) Whole blood

1. Objections to whole blood: large amount of unnecessary or harmful protein (corpuscles, fibrin)

(b) Serum, the liquid resulting after clotting (removing corpuscles and fibrin)

1. Contains major proportion of antibodies
2. Called antiserum, because of antibodies contained in it; occasionally called by name of the most prominent antibody (antitoxin in diphtheria or tetanus antiserum)

(c) Modified serum (see Figs 49 to 51, II)

1. Unnecessary proteins removed by salts, etc. (by ammonia and sodium salts in diphtheria antiserum)
2. Remaining globulins and pseudoglobulins retain most of the desired antibodies
3. Irritating effects (rashes, fever) reduced by such modification (diphtheria, tetanus)

(d) Serum extracts *
1. Watery solutions of antibodies (pneumonia, antiserum extracts)
2. Reduced protein content of such extracts too low for ordinary chemical tests; possible irritation less
3. Absorption more rapid

G. Tests for existence of disease and immunity to disease
(A) Presence of disease organisms in customary region (throat, diphtheria; nasal membrane, meningitis carrier)
a. Identification microscopically: shape (streptococcus); staining (tuberculosis, Fig. 87; diphtheria, Fig. 64, II)
b. Identified by cultural characters: dissolving of blood (pathogenic streptococci, influenza organisms, as in Fig. 2, II)
c. Identified by testing against related serum: typhoid rods clump when mixed with serum from typhoid case (Fig. 78, II)
(B) Conclusions based on changes in white corpuscle counts
a. Corroborating other symptoms; increase expected in pneumonia; decrease in typhoid, influenza
b. Testing improvement during progress of disease, or a series of vaccinations (boils)
(C) Demonstration of specific antibodies characteristic of given disease (agglutinins in typhoid) in blood of patient.
a. Diagnosing disease during onset or early stages, thus expediting benefits from treatment (typhoid)
b. Determining beforehand susceptibility to a given disease (diphtheria antitoxins present or lacking, Schick test, A, Plate I, Vol. II)
c. Identifying carriers, apparently recovered but still harboring disease organisms (intestines in typhoid fever)

*Crystallized antibody extracts have recently been obtained from pneumonia antiserum.

(D) Demonstration of supersensitiveness to specific irritating substance (protein); shown by effects (reddening, temperature rise) out of proportion to minute amount of material inserted (Plate I, Vol. II)

a. Micro-organisms: killed tuberculosis organisms (Von Pirquet skin test)

b. Food or other proteins

(a) Milk, eggs, etc., in food sensitiveness

(b) Pollens, horse or cat hairs, orris powder, etc., in "hay fever" etc.

DEMONSTRATION AND LABORATORY WORK

1. How many of the common communicable diseases listed in Bulletin 436 of the U. S. P. H. Service are caused by bacteria? By protozoa?

2. Locate in the accompanying microscope a white corpuscle which has ingested bacteria, as in Fig. 96, II.

3. Is the white corpuscle change in Pl. II, II one which in ordinary human diseases would lead you to predict recovery?

4. Fresh serum taken directly from inflamed peritoneal cavity in a fatal case of peritonitis (Fig. 29, II). The peritoneal cavity and all tissues are normally sterile; note the immense number of streptococci, mainly in short chains, and the small number of white corpuscles.

5. This slide (Fig. 132) shows the exudate from the nose in a grip-like cold sent into a hospital for diagnosis; the specimen was not incubated, so the slide indicates the actual condition of the fresh exudate.
Why should people who are ill with colds or sore throats refrain from handling food, etc., used by others? Why should milkers wash their hands before milking? Is sneezing dangerous to one's companions? Give another important application or hygienic rule which should be followed by people with external lesions or infections.

6. The paler part (Fig. 133) to the right of this slide (ovary of a dog) is an abscess; note that the cells are wholly disintegrated in this region. To the left are normal cells, showing definite nuclei, etc. (See also Figs. 3 to 6, II.) In some diseases (tuberculosis), a capsule may be formed, surround-

FIG. 132.—Bacteria in nose exudate in a "running cold."

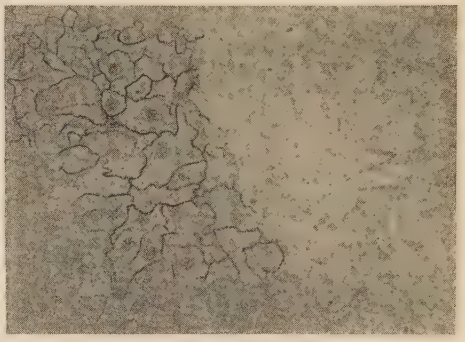

FIG. 133.—The right side shows disintegration of tissue (ovary) due to bacteria.

ing and isolating this disintegrated area, and the patient may entirely recover.

7. Apparatus for injecting antiserum in pneumonia (Fig. 58, II). How is the serum separated from the clotted corpuscles and fibrin? (Fig. 57, II.)

8. Syringes used to inject antitoxins, vaccine, etc. (Figs. 58, II and 122, II). Notice the graduated scale allowing the physician to suit the dosage to the stage of the disease, severity of the attack, etc.

9. Find in Part II illustrations picturing the action of two of the antibodies listed under E. (B) b. of this chapter.

10. Consult the fold-in chart in Part II and describe two differences (1, origin and 2, effect or action) between vaccines and antiserums.

11. Name three vaccines of unquestioned value.

12. What two things in common have all vaccines?

13. After reading the third chapter in Part II, consult the more advanced texts listed at the end of this chapter to determine (1) the animal most often used to produce antiserums; and (2) the diseases in which antiserums are now commonly used.

(See the reference list for Chapter IV as well as the general reference list in the Appendix.)

REVIEW AND STUDY QUESTIONS

1. Name at least one human disease due to each of the following types of parasites: (1) round worms; (2) protozoa with insects as alternate hosts; (3) bacteria; (4) bacteria with insects as alternate hosts; and (5) fungi.

2. What body reactions protect us against disease developing in the body?

3. What is a vaccine? What is the origin of the name? What two characteristics apply to every vaccine, whether live organisms, dead ones or merely their toxins?

4. What is immune blood? What four types of antibodies may it contain?

5. Give four accepted vaccines and the material used as a vaccine in each case.

6. Give four antiserums or immune serums in general use, naming at least one antibody present in each.

7. What is the Schick test?

8. Name two ways in which the actual presence of disease organisms may be proven.

CHAPTER XIII

THE HISTORICAL SETTING OF PRESENT-DAY BACTERIOLOGY

LECTURE AND READING OUTLINE

The following chronological list is designed as a setting for the still growing science of bacteriology, and because of the complex technical character of the whole subject, forms the last chapter of this text instead of occupying its traditional place in the first chapter. Some knowledge of the various phases of microbiology is necessary for comparing the simple beginnings, the development, and the present status of the subject; without it there can be no intelligent appreciation of the wonderful progress that has been made.

Many of the items are not strictly bacteriological, but they help to give the setting, or prepare the way for a later discovery or investigation. While most of the statements record the *first* event or discovery, similar ones are also given if they add materially to our picture of science during the period of the prevailing interest or the trend of the times.

It has not been possible to check up all the dates given here; in most cases they are the dates of publication; but at other times, following custom, the earlier date of discovery or investigation is used, such citations often indicating more correctly the worker's place in the whole field of microbiology.

I. Early theories of disease and origin of life, etc., sometimes correct, as Sanskrit theory of mosquito relation to malaria (6th century, 15th century); usually, however, vague or erroneous, as shown markedly in

Beliefs

—influence of evil spirits, malign gods

—demoniacal theory of disease

—power attributed to charms, sacrifices and ceremonials

—belief in "spontaneous generation" of life (see p. 235)

—common insanitary practices—often raised to rank of ceremonial or religious rites

—influence attributed to astronomical forces (plague, 14th century, due to conjunction of planets—Mars, Jupiter and Saturn)

Medical practices

—humoral theory of disease: diseases due to abnormal pro-

portion of the four body humors or liquids: blood, phlegm, bile and "black bile." Attributed to Hippocrates (about 500 B. C.); persisting in part (practice of "bleeding") to recent times

—fossil bones (ground to powder, alcoholic extracts) in medical treatment (China)

II. Sanitary observances and institutions, varying from purely practical to religious, but all having their effect on the resulting health of the people and the "level of life." Among these might be mentioned

—the gigantic water aqueducts of Italy, France and Spain, some of which—built 2000 years ago (Appian aqueduct, 312 B. C.)—are still in use

—purification methods—Homer, sulphur; Bible, fire

—the sewer systems of Assyria

—the Jewish regulations regarding isolation of lepers, prohibitions with regard to eating of certain meats (animals, such as swine, now known to be particularly susceptible to tuberculosis, etc.) and the draining of blood from the slaughtered animal (delaying decomposition)

—isolated rulings, such as (1276) prohibiting the killing of animals for food except in a designated slaughter house

—legislation concerning disease (isolation, disinfection)

—Chinese inoculation of smallpox (pus on wool threads in nose, etc.)

—methods of food preservation (drying, smoking)

III. Chronological sequence of bacteriological facts and principles and related material

1536—Paré—substitution of milder lotions for boiling oil, hot pitch, etc., used in treating wounds

1546—Fracastorius—theories concerning disease

—true nature of contagion—due to "living material"

—disease transmitted—immediate contact

—intermediate agents

—air

—organisms—of a "glutinous" nature

—capable of reproduction

—made pathogenic through the heat of the animal body

1590—Janssen—first microscope

1659—Kircher—first to see and describe bacteria

—formulated theories concerning infection

—transfer by flies feeding on "excretions of dead and dying"

—wide distribution of infectious organisms; ("worms") seen in "milk, cheese, putrid substances," etc.

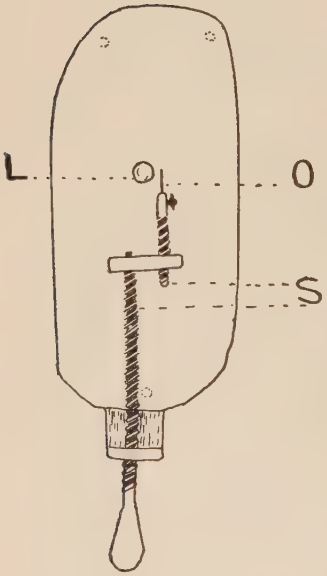

FIG. 134.—One of the one hundred and forty microscopes owned by Leeuwenhoek at the time of his death. This consists of a glass ground into a lens (L) set between two pieces of metal which are riveted together. S indicates screws to assist in focusing objects placed on O. (After Locy: Biology and Its Makers, Holt.)

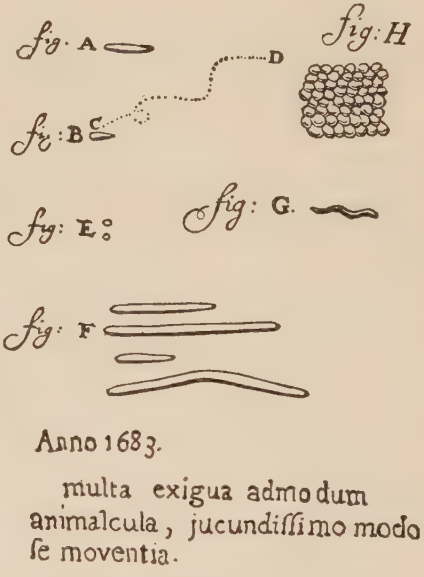

FIG. 135.—The first pictured bacteria, described by Leeuwenhoek in 1683: A, the largest, which was found in "tartar scraped from the teeth and mixed with water"; B, a second type from the same material, which "sometimes spun around in a circle like a top, and sometimes described a path like that shown in C–D." Some "looked like a swarm of gnats or flies dancing about together, E; and some were curved and others were straight, as shown in F."

1675—Leeuwenhoek—first pictured bacteria, described as little animals (animalculæ) seen in rain water, saliva, putrefying material, etc.

1667—Hooke—plant cells first seen (cork) and pictured; cells were thought to be hollow units or "cells"

1688—Redi—first statement of biogenesis: living things from living things only, as opposed to "spontaneous generation"

Spontaneous generation—belief early fixed in common mind
—Even to-day locally shown in such beliefs as "horse-hair worms" from horse hairs
—Aristotle (384-322 B. C.) stated that animals sometimes arise from soil, plants or other animals spontaneously
—Cordano (1501-1576) held that water gives rise to fish and animals
—van Helmont (1577-1644) mice "created from old linen and a few particles of cheese or grain"
1688—Redi—first statement of "biogenesis": living things from living things only
1745—Needham—heated meat effusions in hot water bath; later spoilage "proved" spontaneous generation
1753-6—Linnæus—combined causes of disease, fermentation and putrefaction under "Chaos"
1762—Bonnet—suggested "germs or their eggs" able to resist temperatures used by Needham
1769—Spallanzani—air required for spontaneous generation
1820—Ozanam—opposed live or animate nature of contagion; said it was "not worth wasting time refuting such absurd statements"
1836—Schulze—no spontaneous generation with chemically-filtered air (acid, alkali)
1836—Pasteur and Chevreul—putrefaction does not occur in meat protected from contamination
1839—Schwann—none with heated air
1854—Schröder and Dusch—none with cotton-filtered air
1860—Hoffmann (also Pasteur and Chevreul)—none in flasks opening downward
1861—Pasteur—boiling does not kill all organisms
—putrefaction and fermentation not cause of spontaneous generation, but are themselves due to metabolic activities of micro-organisms
1876-7—Tyndall—no decomposition in dust-free air; proven by tubes left open in box tested with light rays for visible particles (dust, bacteria)
—organisms in decomposing substances always derived from organisms present in air, liquids or vessels themselves

1627–1691—Boyle—predicted that problem of infectious diseases would be solved when fermentation was understood; diseases due to small animals enclosed in the blood

1701—Andry—theory, animalcules the cause of disease

1717–21—Inoculation against smallpox—introduced by Lady Montague into England (1721) from Turkey

1718—Lancisi—theory, animalcules the cause of malaria

1745—Needham—" proved " spontaneous generation
1753–56—Linnæus—the causes of disease, fermentation and putrefaction grouped in class called " Chaos "
1760—Early legislation concerning wheat rust (disease itself known since Aristotle, 350 B. C.)
1762—Plenciz—theories—all infectious diseases due to living micro-organisms
—period of incubation explained by multiplication in body
—specific germs for each disease; supported by differences in incubation period
—air transfer suggested
1764—Hartes—leguminous plants (clover, vetch) enrich the soil
1765—First medical course in the United States instituted
1769—Spallanzani—air necessary for spontaneous generation
1772—Priestley—nitrous oxide (laughing gas) first prepared
1772—Corti—movement in cytoplasm of cell first observed
1774—Priestley—discovered oxygen; name given by Lavoisier; obtained 1773 (but not published) by Scheele; known to Paracelsus [1493–1541])
1779—Ingen-Houss—recognized difference between plant respiration and photosynthesis
1782—Canning of vinegar introduced by Swedish chemist
1786—First classification of bacteria as vibrios: bacillus, lineola, and spirillum
1786—Müller—morphological details regarding micro-organisms (41 species); classified them as *Monas, Proteus* and *Vibrio*
1787—Wollstein—glanders transferred by inoculating (under skin) pus from horse with glanders
1795—Gordon—thought puerperal fever contagious—traced to earlier cases
1796–98—Jenner—vaccination against smallpox with cow pus; opposed by medical authorities as brutal and inhuman
1797—Viborg transferred glanders (pus, urine, saliva) from glanderous horses
1800—Davy—first described anæsthetic power of nitrous oxide
1804—Saussure—carbon dioxide and water combined in photosynthesis by green plants
1804–10—Appert—devised process for canning—corked jars set in boiling water

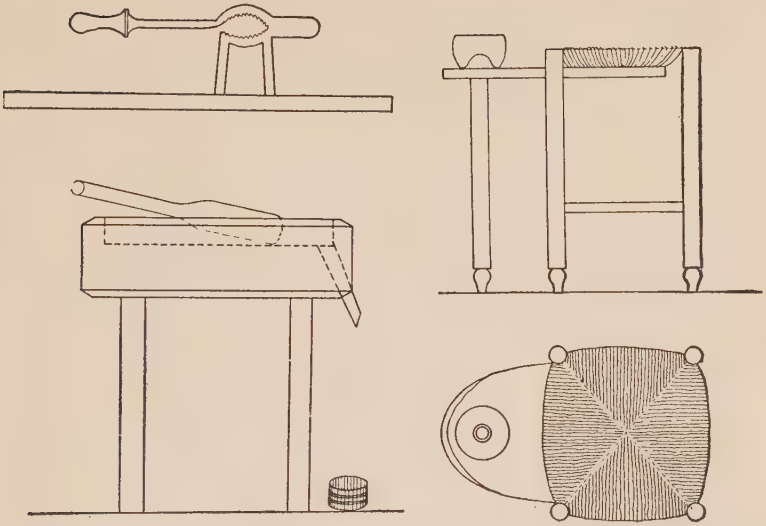

FIG. 136.—Canning apparatus, used by Appert (1804–1810), who first canned foods. The cup on the extension of the stool held the jar while the cork was being wired in; the pincers were used to compress and shape the corks. These corks were later driven in firmly by the mallet; the floor basin caught any overflow from the jars. (Reduced from Bitting's translation of Appert's *Book for All Households.*)

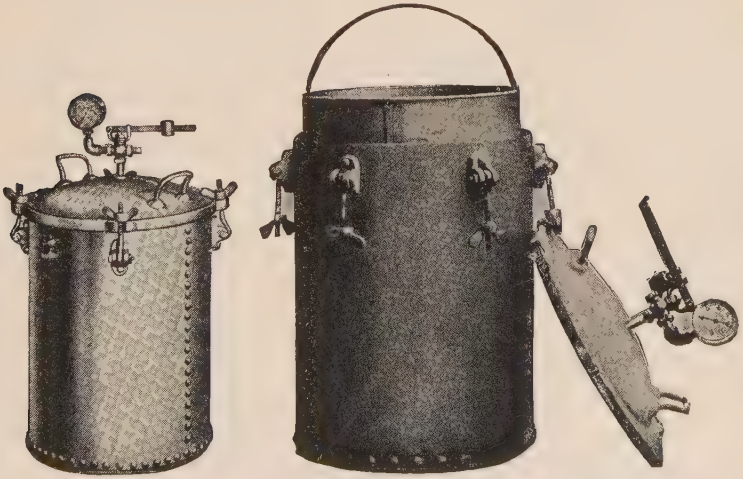

FIG. 137.—The second Appert adapted the autoclave to the processing of foods. The first Appert placed his filled jars in water and boiled them, as in the present "cold pack method." This shows a common type of pressure cooker, essentially a single jacket autoclave (FIG. 93). (Northwestern Steel and Iron Works.)

1807—Prévost—grain rust, a fungous disease
1810—Durand—English patent for canning in glass and tin
1812—Courtois—iodine first isolated—ashes from seaweeds
1813—Magendie and Bouchet—rabies transferred to dogs by saliva of rabid man
1814—Kirchhoff—enzyme action in barley extract described
1819—Daggett and Kensett—canned fish, oysters, etc.
1820—Ozanam—animate nature of contagion too absurd to waste time in refuting
1820—Underwood and Mitchell—fruits canned—glass, tin; shipped long distances, South America, 1821
1828—Peacock—first round worm disease (trichinosis)
1831—Liebig and Soubeiran—chloroform discovered
1831—Brown—nucleus of cells discovered; vital part of cell
1831—Henry—steam used—large scale—for disinfection
1833—Payen and Persiz—first named enzyme or ferment; diastase, changing starch to sugar
1834—Renucci—first human disease organism (mite)—seen in itch
1834—Runge—carbolic acid (phenol) discovered
1836—Schulze—chemically filtered air prevents spontaneous generation
1836—Latour—yeast plant discovered
1836—Schwann—pepsin (protein breaking) enzyme in gastric juice
1837—Pasteur—suggested micro-organisms cause of wound infections
1837—Winslow—first record of canning corn (on the cob)
1837—Bassi—fungous disease of silk worms discovered
1837-38—Latour and Schwann independently—yeast the cause of fermentation
1838—Ehrenberg—achromatic objective
—detailed study of bacteria, protozoa and intermediate forms
1838—Schleiden and Schwann—the cell, or unit of plant and animal structure, a protoplasmic unit
1839—Schwann—heated air prevents spontaneous generation
1839—Berg—thrush, caused by a mold
1839—Schoenlein—favus, a mold, the cause of "honeycomb" disease of scalp

1840—Henle—microscopic organisms probable causes of infectious diseases; to prove it for given disease the organism must be—constantly present in disease
—isolated from infectious material
—alone capable of producing the disease

1841—Fuchs (and Ehrenberg)—blue and yellow milk bacteria investigated

1841—Valentine—first protozoan blood parasite seen (in fish)

1842—Jackson—ether first used as anæsthetic (1846, Morton)

1843—Holmes—puerperal fever contagious; transmitted by attendants

1843—Malinsen—ringworm a fungous infection

1844—Dolland—immersion lens invented

1847—Simpson—chloroform used in surgery

1848—Nott—theory of insect spread of yellow fever

1848—Bell—used steam to disinfect yellow fever infected vessels

1848—Leidy—water from melting ice contains protozoa

1848—Pasteur and Liebig—discussion of fermentation: Liebig, chemical and Pasteur cellular theory

1849—Leidy—bacteria (vibrios) classed as plants
—plants parasitic on animals—intestines of insects and mammals

1849—Pollender—bacteria found in blood of animals ill with anthrax

1850—Burnett—all *Vibrionia* not animals—some belong with plants

1850 (?)—Clinical thermometer first used

1850—Davaine—anthrax in blood and in lesions of infected animals

1851–55—Boussingault—green plant assimilation of nitrates (not nitrogen)

1852—Kückenmeister—flat worm (tapeworm) as human parasite

1852—Perty—observed spores in bacteria

1853—Cohn—bacteria classed as plants

1854—Broad Street well epidemic of cholera worked out

1854—Pasteur—wine investigations begun; heating to remove undesirable organisms introduced commercially 1867–8

1854—Schroeder and Dusch—no spontaneous generation with cotton-filtered air
1856—Perkin—discovered value of aniline in dyes
1857—Nageli—bacteria classed as plants (fission plants)
1858—Murchison—disease due to emanations from putrefying organic matter and to swallowing such matter in food and water
1859—Payen—agar extract obtained from seaweed
1859—Darwin—published *Origin of Species*
1860—Bell—steam disinfection adopted in hospital
1860—Hoffmann—flasks opening downward only—no spontaneous generation
1860—Zenker—trichinosis—due to round worms
1861—Pasteur—some organisms survive boiling
—increased temperatures (110–112 C.) destructive to resistant bacteria
1861—Semmelweis—control of puerperal fever by use of disinfectants
1861—Schultze—protoplasm essentially the same in all living organisms
1862—Pepsin isolated; enzyme or action named earlier (Schwann, 1836)
1862—Pasteur—advanced theory, nitrification of soil due to micro-organisms
1863—Boston—first chartered training school for nurses (Philadelphia 1828 first (?) training school)
1863—De Bary—alternation of plant hosts (for fungi) proven
1863—Davaine—concluded disease result of specific activity (ferment) of bacteria (anthrax)
—inoculated anthrax blood; first bacteria definitely shown to be cause of disease
1865—Pasteur—began investigation of silk-worm epidemic (1849–1868); two causes (muscardine, Bassi, 1837) and "flacherie," a protozoan infection; controlled by egg examination
—spores—reproductive bodies
—specificity of organisms
—high dilution method of obtaining pure cultures

1865—Tuberculosis induced by Villemin—by inoculating rabbits with affected tissue

1866—Woronin—nodules of leguminous plants (clover) contain rod-shaped organisms

1866—von Hessling—believed fungi associated with cheese ripening

1866–71—Micro-organisms demonstrated in wound infections (Rindfleisch, Klebs and others)

1867—Martin—theory—cheese ripening similar to alcoholic, lactic and butyric fermentation

1867—Lister—oxygen not the cause of decomposition, as it does not occur in flasks opening downward only
—first publication on antiseptic surgery (carbolic acid, etc.); previously, amputations 50 to 65 per cent. fatal
—suppuration a kind of fermentation

1867—Crookes—established power of carbolic acid to destroy infectious matter

1867—Hoffmann—formalin (formaldehyde) discovered

1868—Pasteur's method of heating wine used commercially

1868 (1873)—Obermeier—discovered organism of relapsing fever—first pathogenic spirochæte
—inoculated himself with relapsing fever; first human inoculation with *known* organism

1868—Le Fort—weak alcohol used for dressings

1869—Smith—antiseptic value of aniline dyes

1870–72—Bacteria seen in white corpuscles

1870—Abbé—invented condenser for microscope

1871—Klebs—anthrax blood not pathogenic after filtration
—bacteria cause wound infections
—used hen's eggs as pure (!) culture medium

1871—Bacteria stained—Weigert and others (carmine, etc.)

1871—Burdon-Sanderson—freezing does not always kill all bacteria

1871—Bacteria present in lymph in erysipelas

1872—Pasteur—specificity of bacteria—pigmented forms

1872—Cohn—first use of the term bacterium—systematic classification of miscoscopic plants: micrococcus, bacterium, bacillus, vibrio, spirillum, spirochæte

1873—Klebs—bovine tuberculosis transferred by feeding milk

1873—Schlösing and Müntz—theory, micro-organisms cause nitrification in soils
1874—Armauer-Hansen—leprosy organism discovered
1874—Traube and Gescheidel—freshly drawn blood destroyed bacteria
1874—Panum—vague theory of white corpuscle action
1874—Hall—milk obtained sterile condition from cow
1875—Lösch—tropical dysentery due to an ameba
1875—Cohn—spores but a phase in the life of certain bacteria (anthrax)
—spores more resistant to heat, drying and chemicals
1876—Koch—splenic fever due to anthrax bacteria
1876—Tyndall—origin of bacteria in decomposing substances always traceable to air, substances or containers
1876—Nencki—ptomaines from decomposing flesh first analyzed
1877—Blount—sunlight injurious effect on bacteria
1877—Bollinger—"lumpy jaw" of cattle due to fungus (actinomyces)
1877—First protective serum—Raynaud—on calf (1878?)
1877—Woronin—first protozoan disease of plants (cabbage)
1877—Aniline dyes as bacterial stains—Weigert and Ehrlich
—differential stains—Koch, bleaching with acid; Gram, bleaching with alcohol
1878—Klebs—inoculated human disease in monkeys (syphilis)
1878—Duclaux—first study of micro-organisms in connection with cheese-making
1878—Carter and Koch—transferred relapsing fever of man to apes
1878—Sëdillot—term microbe first used; also by Metchnikoff(?)
1878—Burrill—first bacterial disease of plants—pear blight (Prillieux—bacteria seen in wheat kernels)
1879—Miquel—thermophile bacteria, growing at 65–70° C., active in fermentation
1879—Neisser—gonococcus seen
1879—Strasburger—nuclei derived from preceding nuclei only
1879—Frank—nodules absent on leguminous plants (beans, clover) grown in sterilized soil

1879—Pasteur—bacterial origin of puerperal fever proven
1880—Zeiss objective perfected
1880—Toussiant—heated blood of anthrax infected animal used to protect sheep (vaccine: weakened organisms)
1880—Pasteur—fowl cholera bacillus isolated
—immunity by attenuated virus (age) : fowl cholera, also anthrax
1880—Laveran—malaria—protozoa in blood discovered
1880—Eberth—typhoid organism discovered (Klebs, 1881)
1880—Pneumonia seen by Sternberg and by Pasteur
1881—Hansen—yeast—single cell method of securing pure cultures; solid media before Koch (?)
1881—Koch—solid media for obtaining pure cultures; purity of all cultures and validity of all names uncertain before
1881—Pasteur—rabies produced by inoculation with brain or spinal cord of rabid animals.
1881—Koch and others—destructive effect of steam on bacteria
—streptococcus seen in pus and abscesses
1882–85—Millardet and Prillieux—chemical sprays to control plant diseases; lime and copper sulphate (Bordeaux mixture) successful (copper against smut discussed Prévost, 1807)
1882—Attenuation of bacteria by chemicals—Roux and Chamberland
1882—Koch—tuberculosis isolated. Henle's theory reformulated as four laws for proving a given organism cause of a specific disease
—organism present in all cases of a given disease
—organism isolated in pure culture
—pure culture produces the disease in inoculated animals
—organism obtained again in pure culture
1882—Pasteur—rabies cured by attenuated form of organism
1882—Loeffler and Schütz—glanders—isolated from nasal discharge of horse
1882—Metchnikoff—bacterial destruction by white corpuscles determined
1882—Krukowitsch—ozone destructive to putrefying bacteria

1883—Klebs (Loeffler later, pure culture)—diphtheria organism discovered
1883—Koch—cholera isolated
1883—King—theory malaria due to mosquito bites
1883—Fehleisen—erysipelas—a streptococcus infection (seen earlier, 1882, in abscesses)
1884—Leber—chemical attraction between white corpuscles and bacteria
1884—Neisser—tetanus isolated; disease transferred by inoculation with affected blood
1884—Nicolaier—tetanus—produced by inoculation with garden soil; Carle and Rattone (earlier?) by inoculating rabbits with pus
1885—Sternberg—demonstrated disinfecting value of chlorine
1885—von Fodor—saprophytic bacteria destroyed when injected into blood vessels
1885—Bumm—gonococcus—isolated, cultivated on coagulated human blood serum
1885—Selmi—first named ptomaines (from decomposing flesh)
1886—Richet and Héricourt—immune serum (from animals inoculated with staphylococcus) transferred to rabbits
1886—Salmon and Smith—bacterial toxins first tried to secure immunity (hog cholera)
1886—Richardson—ether spray used for local anæsthesia
1886—Bergman—steam sterilization of dressings, instruments
1887—Weichselbaum—meningitis—pure culture obtained from cerebro-spinal exudate
1887—Bruce—Malta fever organism discovered
1888—Beijerinck—nodule organisms are bacteria (See 1866, Woronin)
1888—Nuttall—bactericidal effect of blood serum (later, also Büchner, Flugge)
1888—Winogradsky—nitrifying bacteria (Nitrosomonas) in soils; grown without organic matter
1888–89—Roux and Yersin—toxin of diphtheria discovered
1889—Kitasato—tetanus—pure culture—anaërobic methods developed
1889—Smith and Kilbourne—cause of Texas fever—a protozoan

(renamed, Piroplasma, 1895); ticks and cattle alternate hosts

1890–93—Behring and Roux (independently)—antiserum (antitoxin) helpful against diphtheria

1890—Smith—sterile tissue as culture medium

1890—Sedgwick—first municipal water filter in America (Lawrence, Mass.)

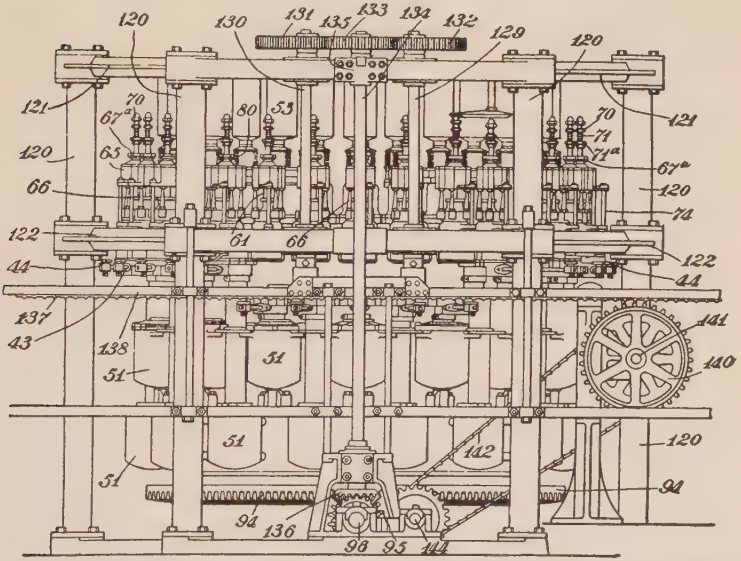

FIG. 138.—A complicated canning apparatus (Patent 1141239, 1915), which has a series of processing chambers (51), each containing but a single can. Since steam, besides filling the chamber as usual, is injected into each can at 280° F., to 350° F., this canning process may take but a few seconds. (Courtesy of the National Canners' Association.)

1890—Behring and Kitasato—immunity against tetanus by antiserum

1891—Roux—immune blood of other animals of therapeutic value to man (diphtheria)

1891—Ehrlich—animals may be immunized against other than bacterial toxins (ricin from castor oil)

1891—Kitasato—toxin of tetanus discovered

1892—Pfeiffer—influenza bacillus isolated

1892—Behring and Kitasato—immune serum (tetanus) used to protect rabbits against infection

1894—Bubonic plague bacillus—Kitasato; also Yersin

1894—Pfeiffer—blood immune animals bactericidal for specific organism

1894—Calmette—antiserum formed against snake venom

1895—Russell—first bacteriological study of canning

1895—Diphtheria antitoxin—used by New York City Department of Health

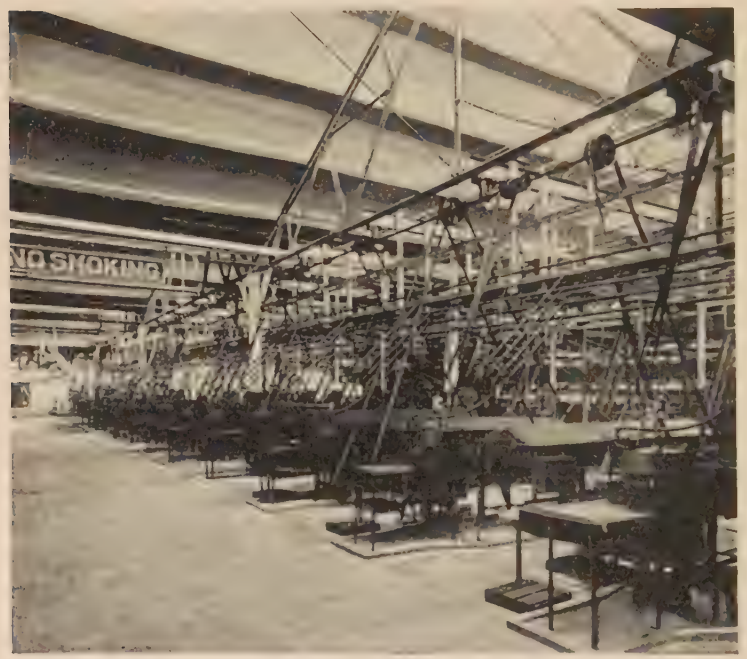

FIG. 139.—Syruping machines with exhaust boxes used in pineapple canning. Compare the complex apparatus in this and the preceding figure with Appert's original canning equipment (Fig. 136). (Courtesy of the National Canners' Association.)

1895—Ross—malaria organisms—crescent stages from human blood fed to mosquitoes underwent cycle changes in stomach

1896—Gruber and Durham—agglutinin against cholera and colon organisms demonstrated in immune blood

1896—Widal—agglutinins used as test for specific diseases (typhoid)

1896—Wright—first vaccine with dead bacteria only (typhoid)
1897—Precipitin by Kraus—plague and cholera
1897—Steam pressure used in canning
1897—Büchner—enzyme "zymase" extracted from yeast cells

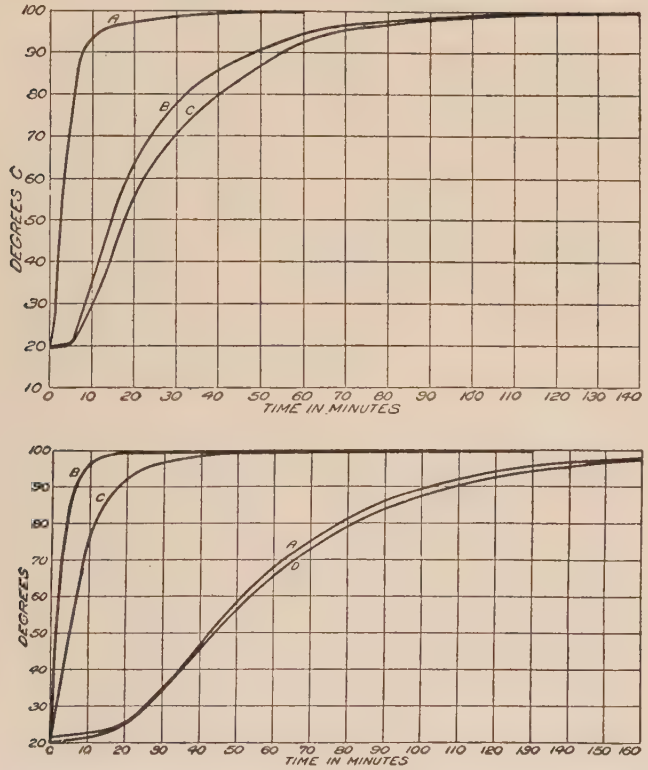

FIG. 140.—Time and temperature requirements are affected by differences in density (tightness in packing, character of substance).

The second illustration shows how much longer it takes to heat a can at the second heating when the watery liquid has become thickened by the softening tissues. Shaking devices for moving the liquid in the cans shorten remarkably the time necessary to secure sterilizing temperatures in the can when the contents are thick or mushy. (After Magoon and Culpepper, U. S. Dept. Agriculture.)

1898—Richet and Héricourt—anaphylaxis (dogs) with second injection of serum (eel)
1898—Loeffler and Frosch—filterable virus (foot and mouth disease)

1898—Bordet—lysins are formed against foreign blood cells

1898—Shiga—dysentery bacillus isolated, (1898–1903—various types by others)

1898—Roux and Nocard—pleuro-pneumonia organisms cultivated—collodion sacs—peritoneal cavity

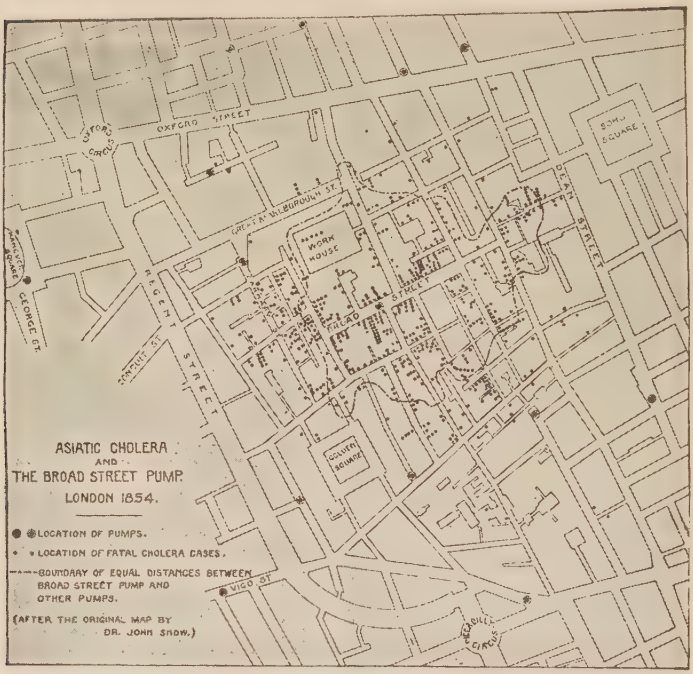

FIG. 141.—The first water epidemic plotted out to determine the origin of the infection. In this case the London cholera epidemic of over 700 deaths was traced to the Broad Street well. Dr. Snow found that most of the victims who lived nearer other wells (see wavy line marking mid-distances) had drunk Broad Street well water while visiting in that area, etc.

1900—Koch and Celli—quinine used in living subject to kill malaria

1900—Yellow fever —experimentally produced in man by Reed, Carroll, Lazear and Agramonte—due to a filterable virus

1901—Beijerinck—bacteria (*Azotobacter*) fix free nitrogen

1902—Bordet and Gengou—complement fixation reaction discovered

1902—Gorgas—control of disease by sanitation—yellow fever, Havana

1903—Protozoa cultivated—Novy, McNeil

1903—Büchner—enzymes extracted from acid-forming bacteria (lactic and acetic)

1903—Wright and Douglas—opsonins

1903—Negri—protozoan (?) bodies in central nervous system of rabid animals

1904—Anaphylaxis—Smith—definitely established; guinea-pigs, with second injection of horse serum

1906—Wassermann, Neisser and Bruck—applied complement fixation in test for syphilis

1905—Syphilis stained—Schaudinn and Hoffmann

1906—Wolff-Eisner—pollen as cause of "hay fever" first suggested

1907—von Pirquet—anaphylactic reaction with killed tuberculosis as test for disease

1908—Flexner—cause of epidemic cerebral meningitis

1911—Noguchi—pure cultures of syphilis (agar plus tissue)

1913—Schick—test for susceptibility to diphtheria, by injecting toxin

1913—Flexner and Noguchi—infantile paralysis stained and cultivated; then smallest known organism (bacteria or protozoa still disputed)

1913—Behring—"toxin-antitoxin" combination as diphtheria vaccine

1914—New York City—ordinance all milk pasteurized except grade A

STUDY AND READING SUGGESTIONS

1. What discoveries in chemistry laid the foundation for bacteriology? In physics? In biology, *e.g.*, cell structure?

2. What investigations in chemistry and bacteriology combined to advance progress in surgery?

3. Pick out one fact or discovery which would have had a profound effect upon microbiology if known (twenty, fifty years) earlier.

4. What separate discoveries or investigations were necessary before pasteurization of milk could become a recognized procedure? Before a yellow fever vaccine could be prepared (Noguchi, 1919)? Before the Panama Canal Zone could be freed from the yellow fever scourge by Gorgas?

5. Prepare three other questions with beginnings reaching back at least a hundred years.

6. When did theory and speculation give way to established facts and principles concerning specific micro-organisms?

7. What events led to the present idea of the active part played by the human body in resisting disease?

8. Read in some of the historical and biographical references cited below, gleaning items that help make bacteriology and its advances more

FIG. 142.—Pear blight was the first-known bacterial disease of plants (*Erwinia amylovora*). (After Smith: Bacterial Diseases of Plants, Saunders.)

vivid and real—items that carry the "human touch"; *e.g.*, the mishap through which Koch's assistant stumbled upon the differential staining of tuberculosis; the accident which led Pasteur to use attenuated organisms as vaccines and developed the whole theory of modern vaccines; and the pioneer work of Trudeau and heroic services of such men as Reed and Lazear.

9. Name the date for each of the following discoveries: The first protozoan disease of animals. Of man. The first bacterial disease of animals. Of man. Of plants.

10. What have been the main lines of bacteriological interest since 1914? (Leaf through *Abstracts of Bacteriology* to help in answering this and the following question.)

11. What diseases due to micro-organisms are occupying most of the attention of investigators to-day?

12. Name one important disease organism which has been discovered since 1914; one valuable vaccine; one accepted antiserum.

Practically all of the standard texts listed in the Appendix contain an introductory chapter showing the historical background of microbiology. Often, the introduction of each chapter contains similar information; and much can be gleaned from the discussion of the various subjects, such as vaccination, typhoid fever, disinfection or water purification. Formal addresses, such as Chemistry and the Recent Progress

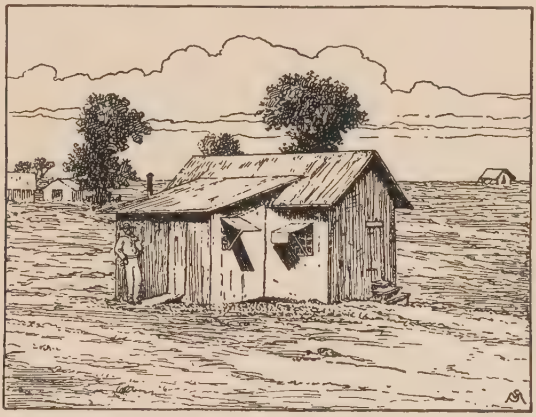

FIG. 143.—Camp Lazear, the shack where Reed contracted yellow fever and Lazear lost his life in demonstrating that the Stegomyia mosquitoes which had bitten yellow fever patients could carry the disease. (Greenberg's Elementary Biology: Ginn & Co.)

of Medicine, by Steiglitz (Johns Hopkins Bulletin, 1924), will be helpfull also. Among the books most useful to first-year students are the following:

Locy: *Biology and Its Makers,* Holt, New York, 1908.
Duclaux: Pasteur, *The History of a Mind* (translated by E. F. Smith and F. Hedges), Saunders, Phila., 1920.
Sedgwick: Principles of Sanitary Science, Macmillan, N. Y., 1922.
Trudeau: *An Autobiography,* Doubleday, Page, New York, 1916.
Vallery-Radot: *The Life of Pasteur* (trans. by R. L. Devonshire), Doubleday, Page, New York, 1914.

APPENDIX

MEDIA

It is now possible to buy in dry form preparations which, when diluted with water, will yield ordinary broth, agar or gelatine at given designated acidities. This greatly simplifies the routine work of a laboratory, but for the beginning student there are real values accompanying first-hand knowledge of proportions and preparation, and the following simplified directions may be of value.

Substitutes for Meat Extract Broth.—Where bacteriological media is needed but once or twice in a school year (demonstration work in high schools, etc.),* it may not always seem desirable to purchase the usual commercial substances, especially the rather expensive meat extract or the large packages of peptone. Under such conditions good growth may generally be obtained with either of the two media described below.

Bouillon Cube Substitute.—Bouillon cubes may replace meat extract and salt. The proportions will then read 1½ to 2 bouillon cubes and 10 gms. of peptone to one liter of water. A still cheaper substitute follows:

Canned Soup Substitute.—To one half pint can of clear meat bouillon, add 4 to 5 cans of water, 1 tablespoonful of cane sugar (or molasses) and one-half teaspoonful of table salt. Warm slightly (until thoroughly dissolved), filter through filter paper, sterilize, etc., as described in Chapter VI. For solid media, add the usual proportions of agar or gelatine, as described in that same chapter.

Neutral Red Broth.—Since this medium is used mainly to determine the ability of a given organism to use lactose (forming gas, acid), pure lactose should be used.

To the desired amount of ordinary broth add 1 per cent. lactose and 1 per cent. of a saturated watery solution of neutral red dye. Tube in fermentation tubes and autoclave.

Litmus Milk.—Use only fresh milk of the best quality (and which has therefore a low acidity).

Siphon off the cream or siphon the milk out from under the cream, as the white cream layer may be confused with the white color which develops when litmus is "reduced." To fresh milk (but slightly acid

*Occasionally, in an isolated locality, expensive materials may be needed in much smaller amounts than those listed in the catalogs of wholesale dealers; a card addressed to the author will be sufficient to secure the address of a retail dealer who will supply small amounts of the desired substances at reasonable prices.

when tested with litmus paper, or not more than 1.5 acid when titrated with phenolphthalein), add a 5 per cent. litmus solution, obtaining a pinkish-blue pastel shade. Tube in sterile tubes and Arnold on three successive days. Chill, on removing from the Arnold, to restore the color, and leave at room temperature until the next heating.

Litmus milk in dry form may also be obtained; in preparing this it is only necessary to add water and then sterilize.

Starch Medium.—To any desired amount of ordinary agar add 1 per cent. soluble starch, heat in a water bath or Arnold for 40 minutes, shaking (or stirring) frequently to distribute the starch throughout the agar. Tube without filtering, and autoclave.

To test starch digestion, pour plates as usual, and when "set" streak out the organism and incubate until a characteristic growth occurs, (5-7 days if necessary). Flood the surface with a saturate solution of iodine * in 50 per cent. alcohol; the breadth of the clear zone around the growth will indicate the extent of starch digestion.

Potato.—Scrub the whole potatoes thoroughly, and rinse in running water to remove resistant soil organisms. Cut the potatoes into long, straight-sided, square-ended pieces, making the short diameter about 1/5 less than the diameter of the test tubes to be used. Cut off slantwise the upper half of one long side to give a convenient slanted surface for inoculation (Fig. 80). Then soak in running water over night, to overcome the natural acidity of the potato and remove all loose starch grains along the cut edges, which should be left sharp-cut. (If preferred, stand instead in a generous amount of a neutralizing solution [1 to 1000 sodium carbonate] for several hours, shaking occasionally). Tube, adding water to the level of the lower edge of the slanted surface, and autoclave. (See pp.105-6.)

Brilliant Green Agar.—This is practically ordinary agar to which is added a very small amount of "brilliant green" dye—in proportions of 1 to 250,000, 330,000 and 500,000. The inhibitive action on *coli* can be demonstrated by using it in the proportions given for a series of streak or pour plates of *coli*. Other refinements of this method are necessary in making typhoid diagnoses (fecal examination); but since beginners, for whom this course is designed, will not be using pathogenic organisms, such details are not included in this chapter.

Endo's Medium.—This is sometimes necessary to furnish supporting evidence of the presence of *coli*. In preparing it, even from the directions in more advanced texts, it is well to keep in mind the three groups of substances represented in this differential medium: (1) ordinary nutritive agar; (2) lactose, for the *coli* organism; and (3) the

* Much weaker solution (one-half or one-third this strength) is often sufficiently strong to give a satisfactory contrast of undigested (blue-black) and digested (transparent) starch areas.

decolorized fuchsin used because of the color change produced in it by *coli*. The three substances are mixed when needed as the color returns gradually on standing, even in sterile plates. The simplest, though not the most differential medium, is prepared as follows:

(1) Bottle ordinary meat extract agar (slightly alkaline to litmus or about pH 7) in 100 c.c. amounts and sterilize.

(2) Tube in sterile tubes, 1 gm. amounts of lactose. Handle with care (weighing on sterile paper, etc.) to lessen the chance of contaminating organisms. When needed, pour one gram into the melted agar (100 c.c.).

(3) To each 100 c.c. bottle of melted agar and lactose add the decolorized fuchsin. Use 10 c.c. of (freshly prepared) 10 per cent. watery solution of sodium sulphite and 1 c.c. saturate alcoholic solution of fuchsin heated in the Arnold for 20 minutes.

Pour into plates, and dry somewhat (incubator for 30 minutes, lids lifted) before streaking out the coli-containing culture. A deep red color in the growth is characteristic of *coli*.

STAINS* AND SOLUTIONS

Iodine

Make a 10 per cent. stock solution of iodine crystals, 10 gms. of iodine to 90 c.c. of water. (Filter if tap water is used.) Dilute one-half (to a 5 per cent. solution) for staining yeast cells and starch grains.

Iodine-eosin

Potassium iodide	2 gms.
Eosin	1 gm.
Water	250 c.c.

Filter if tap water is used. Useful in staining yeast cells.

Methylene blue

Saturated alcoholic solution of methylene blue	30 c.c.
Distilled water	100 c.c.
Mordant: potassium hydroxide, 1 per cent. solution	1 c.c.

Carbol-fuchsin

Saturated alcohol solution of fuchsin	5 c.c.
Carbolic acid, 5 per cent. solution of pure crystals (see solutions following)	40 c.c.

Carbol-gentian violet

Same proportions as for carbol-fuchsin

*The Society of American Bacteriologists is endeavoring to help laboratories to secure standardized stains, etc., and issues for the use of producers special labels of approval to be used for stains which have been tested by a committee of that Society.

ANILINE-GENTIAN VIOLET

(Sometimes preferred to carbol-gentian violet for Gram's stain; the former, however, keeps better)

I. ANILINE WATER

Distilled water	98 c.c.
Aniline oil	2 c.c.

Shake thoroughly and filter until clear.

II. Saturate alcohol solution of gentian violet	5 c.c.
Aniline water (I above)	15 c.c.

GRAM'S SOLUTION

Iodine	1 gm.
Potassium iodide	2 gms.
Distilled water	300 c.c.

Keep in the dark; make fresh every 2 or 3 months.

GRAM'S STAIN

Stain the smear with gentian violet (5 min.), wash in water; set the stain with Gram's solution until the smear is black (purplish black) (1½ to 2 min.); decolorize with 95 per cent. alcohol until pearl gray (5 min.?); wash, dry and mount in balsam. Note the controls used in Fig. 88.

COUNTER STAINS

For tuberculosis (carbol-fuchsin); after decolorizing, wash in water and stain with ordinary methylene blue for one minute; wash, etc. as usual.

For Gram's stain (gentian violet): after decolorizing stain one-half (to one) minute with 0.5 per cent. solution of safranin.

TUBERCULOSIS STAIN (Acid-fast stain)

(1) Dry and fix the smear (tuberculosis culture, sputum) as usual.

(2) Rim the smear with a wax pencil and flood it with carbol-fuchsin. Turn a flame down toward the stain until it steams; repeat often enough to keep the stain warm (steaming slightly) for 5 minutes.

(3) Wash in water and decolorize (p. 119) until the red color disappears (smear colorless or faintly pink).

(4) Wash in water and drain.

(5) Counter stain with a saturated watery solution of methylene blue, (or ordinary methylene blue for one-half minute.

(6) Wash, dry, and mount as usual.

India ink

Ordinary India ink, as purchased at stationery shops, usually contains bacteria. Heating it on 5 to 8 successive days at 80° C. sometimes yields a sterile ink without making it lose the smooth homogeneous character so desirable as a background for micro-organisms. Higher temperatures are, of course, more effective in bacterial destruction, but almost certain to cause the ink to "separate." Patented brands of India ink for bacteriological purposes may be obtained at the usual supply houses, but even these are not always free from micro-organisms. When heating to remove bacteria, it may be wise to transfer the ink to several small tubes; when sterile, seal the top with paraffine or plasticine and open single tubes as needed, discarding a tube if it becomes contaminated. Control smears (India ink alone) should be made to insure that the ink is sterile.

Jenner's stain

This is a combined fixative and stain, with good keeping qualities, if kept corked.

Eosin (0.5 per cent. in methyl alcohol) ..... 125 c.c.
Methylene blue (0.5 per cent. in methyl alcohol) .... 100 c.c.

Dry smears as usual and flood with the stain, covering to prevent too great evaporation. If possible, float the cover-glass face down in the stain (or when staining on slides, immerse the slide in the stain). Stain 3 minutes, wash the smear in a *little* water and mount in balsam for observation.

This is a good differential stain for bacteria in blood or other tissues (red corpuscles, red; all nuclei, blue; and bacteria, blue).

Carbolic solutions

I. For stains: 5 per cent. solution

Dissolve pure carbolic crystals (stand bottle in beaker in water bath, to prevent loss if bottle cracks).

Stand beaker thus warmed on scales and pour out desired weight of the liquefied carbolic acid.

Distilled water, 95 c.c. (approximately 100 c.c. will do) for each 5 gms. of carbolic.

Warm the water slightly to prevent re-crystallization of the acid (or add to the dissolved crystals a little warm water) and then mix with enough room-temperature water to make the total volume.

II. For disinfection

Since the aim is to secure at least 5 per cent. of the acid in solution, volume measurements may be used throughout;

measure the liquefied crystals in a warm graduate and add 100 c.c. of warm tap water for each 5 c.c. of carbolic.

LITMUS SOLUTION

Litmus, purchased in dry form (powder, cubes) varies greatly in strength and purity. Unfortunately the producer's name is not always a guarantee. See footnote on p. 254.) When low in color value, such large proportions may have to be added as to change materially the food proportions and acid reactions of the media. (Because of the high cost of litmus, it may be best to devise some way of using weak preparations; *e.g.*, in making litmus agar, starting with but half the usual quantity of water, and adding the litmus solution for both color and water.)

Litmus solution (5 per cent.) is prepared by adding tap water to the litmus and heating the mixture in the Arnold for 2 hours, shaking frequently to give better opportunity for the dissolving action. Filter, bottle in suitable amounts, and Arnold to prevent spoilage. (The autoclave often breaks down the litmus to a dirty brown substance of no differential value.)

PHENOLPHTHALEIN

Since it is necessary to use 95 per cent. alcohol to dissolve phenolphthalein, determine beforehand the total volume of the solution needed; dissolve the phenolphthalein in the 95 per cent. alcohol and then add distilled water to make the required volume and percentage of solution. Phenolphthalein is usually used in one-half per cent. solutions in 30 or 50 per cent. alcohol.

As one gram is the smallest amount that can be measured with any degree of accuracy, weigh out one gram of the powder, which will yield 200 c.c. of a 5 per cent. solution, and place it in a carefully washed bottle. Then determine the amount of 95 per cent. alcohol needed. For 200 c.c. of a 50 per cent. solution we need 105 + c.c. of 95 per cent. alcohol, (95 : 50 :: 200 : 105 +). Therefore dissolve the gram of phenolphthalein in 105 c.c. of 95 per cent. alcohol; when thoroughly dissolved add to it the necessary balance, 95 c.c. (200 — 105) of distilled water.

SODIUM HYDROXIDE

(See Titration with Phenolphthalein (p. 259) for making normal sodium hydroxide.)

Because of the difficulty in breaking these air-liquefying sticks readily into the exact weights desired, it is better to take any piece that approximates the necessary weight and adjust the *water* bulk to that weight.

For example, for 200 c.c. of a 5 per cent. solution of sodium hydroxide, we should require 10 gms. of sodium hydroxide; if the nearest weight of sodium hydroxide available is 11.5 grams, instead of 10 grams, use the 11.5 gm. piece, placing it in a bottle and adding to it the right amount of water to make a 5 per cent. solution. Then, instead of 10 gms. sodium hydroxide in 200 c.c. of solution, we shall have 11.5 gms. in 230 c.c. for (10 : 11.5 :: 200 : 230). To the 11.5 gm. piece of sodium hydroxide we shall therefore add 218.5 c.c. or 218.5 gms. of water (230 — 11.5 = 218.5).

Since this solution unites readily with CO_2 of the air, store in a bottle approximating the liquid bulk; keep the bottle corked, and discard the solution when it shows cloudiness or precipitates.

POTASSIUM HYDROXIDE

See Sodium hydroxide preceding. Used (in 1 per cent. solutions) as a mordant; see methylene blue direction, under Stains.

LITMUS AND PHENOLPHTHALEIN AS INDICATORS

Litmus.— Paper impregnated with an acid-litmus or an alkali-litmus solution has long been used in testing the acid or alkaline character of a substance. The main objections to the use of litmus as an indicator are that (1) its neutral point is not so near true neutrality as that of other available color indicators, and (2) the litmus color changes are not so sharp or so delicate as the changes obtained with other indicators.

Nevertheless, for preparing broth and agar for ordinary class room demonstrations showing the prevalence of bacteria (air-exposed plates) etc., especially with such simple media as the substitute preparations described on p. 252, it will be convenient to use litmus to determine in a general way the acid-alkaline character of the medium. Medium that is neutral or slightly alkaline to litmus is usually preferred. However, if the medium is but *slightly* acid, good results are usually secured. (See *Correcting Acidity.*)

Correcting Acidity.—In making media, if correction is necessary, it is usually because the medium is too acid. A strong solution of sodium hydroxide (4-5 per cent.) will be found best for this purpose;* a weak solution changes the food proportion too greatly. If testing with litmus, pour out a fraction of the medium found to be too acid, and add to it small amounts of sodium hydroxide until the desired neutral or slightly alkaline condition is obtained. Then use the pro-

* With phenolphthalein a *normal* solution is used, for, as implied under Correcting Acidity, we can calculate how much should be added. With litmus it is almost guesswork. A normal solution of (pure) sodium hydroxide is a four per cent. solution.

portion thus determined in correcting the rest of the medium. In this way, if a mistake is made and too much alkali added, the untreated balance is available for remedying the error. (Otherwise, acids have to be added to correct the excess alkali.)

After correcting a medium by an alkali addition, heat the medium for an hour in a water bath; otherwise precipitates may occur in the tubed material. Filter after such heating; then tube and sterilize as usual.

With phenolphthalein as an indicator, the amount of super-acidity can be quantitively determined. (See Phenolphthalein.) In correcting, nevertheless, work with a margin for safety; measure out the estimated amount of normal alkali needed, and add it fraction by fraction, titrating at least once before the full amount has been added. There are "side-reactions" in such situations, and should the medium remain too acid even after the amount estimated is added, calculate the further amount needed and proceed as before. Heat in a water bath or Arnold before tubing, as recommended above.

Titration with Phenolphthalein.—Although the phenolphthalein method has been quite widely replaced by the newer H-ion determinations, it will continue to be used in many of the smaller laboratories which have only occasional use for bacteriological media, but prefer something more delicate than litmus as an indicator.

Phenolphthalein is colorless in neutral and acid solutions, and pink to deep raspberry red in alkaline solutions. A drop of phenolphthalein, therefore, will indicate at once whether a given solution is acid or alkaline.

When a substance is thus shown to be acid (colorless when phenolphthalein is added) we can further determine how acid it is by seeing how much alkali of a known strength is necessary to cause a pink color to appear. If, on the other hand, a substance is already alkaline, we can measure its alkalinity by seeing how much of a known acid is necessary to neutralize the alkali and make the pink color disappear. For this, we use standard solutions—"normal solutions"—made up on the basis of *one gram of ionizable hydrogen or its equivalent* in each liter of water. In hydrochloric acid (HCl) solutions this means one gram of hydrogen (H) in each liter; in sodium hydroxide (NaOH) solutions this means one gram of hydroxyl (OH). This gram per liter is obtained by adding amounts based upon the atomic structure. For example, with NaOH, every time we add one OH unit we must add one unit of sodium (Na). The atomic weight of hydrogen is 1; of oxygen 16; and sodium, 23. To add enough sodium hydroxide to furnish 1 gram of hydrogen, we must add 16 grams of oxygen and 23 grams of sodium, or a total of 40 grams to a liter. Equal amounts of such

normal solutions will balance or neutralize each other. Therefore, an acid solution which demands 5 c.c. of a normal solution of alkali to cause it to turn pink must contain 5 c.c. of normal acid. In this way the amount of acid present is measured or titrated.

Workers have selected 100 c.c. as the unit of volume. In actual laboratory practice, however, a much smaller bulk of milk, broth, etc., is used for titration—5 c.c., and this is diluted in 45 c.c. of distilled water. Phenolphthalein is then added. If no pink color shows, measured amounts of a normal alkali are then added drop by drop from a special graduated pipette (burette) until the pink color develops. Much more delicate results are obtained if the normal alkali is diluted (20 times, ordinarily, $^{n}/_{20}$).

If the acid in the 5 c.c. amount was neutralized by 28 c.c. of a $^{n}/_{20}$ alkali,

the acid in 100 c.c. (20 × 5) would be neutralized by 2.8 c.c. of a normal alkali;

and the solution contains 2.8 normal acid in each 100 c.c.

In making media we need not only to determine the present acidity (or alkalinity), but usually to correct it as well. (See Correcting Acidity.)

If the medium tested contains 2.8 c.c. acid per 100 c.c.,

and the medium should contain but 1.0 c.c. acid per 100 c.c.,

it contains an excess of 1.8 c.c. acid per 100 c.c.; and

enough alkali or 1.8 c.c. normal alkali for every 100 c.c. of substance should be added to reduce the original acidity to the required acidity, or 1.0 per 100 c.c.

HYDROGEN ION CONCENTRATIONS AND INDICATORS

BY ANNE DIX

The life processes of bacteria and other micro-organisms are very complex, in fact, almost as complex as they are in man. They, also, are affected by many varying conditions of temperature, humidity, oxygen and food. Hence, there is need to make for them as many standard conditions as possible. Considering the food of micro-organisms as one problem, we find in it many complications when we try to reduce it to certain definite arbitrary standards. The meat basis that is used for most culture media, and the various makes of peptones as well, are naturally more or less acid in comparison with true neutrality. It is necessary, therefore, to adjust the reaction of the media to the most suitable point for the optimum growth of the special organisms one is trying to grow.

The question of acid reaction of media especially has long been a problem to the bacteriologist. There are several methods of determining the reaction of a medium: such as by litmus and titration with phenolphthalein; but the determination of its actual acidity, that is, the amount of free hydrogen present in the solution, requires an understanding of the ionic theory. According to this theory every salt, acid or base in aqueous solution breaks down to some extent into electrically charged "ions." Water, also, has this property of separation into its component parts, *i.e.*, H and OH, but only to a very slight extent—only about one hundred millionth of one per cent. of pure water really dissociating into ions. The H-ions carry a positive charge of electricity and have the characteristic properties of any acid, such as sourness. The OH-ions carry a negative charge and give the properties common to bases, such as the smooth slimy feeling to the touch and the brackish taste. In pure water the H-ions and the OH-ions neutralize each other; therefore water has neither acid nor alkaline properties to our usual senses. If, however, a drop of hydrochloric acid (HCl), or in fact, any acid, be added to distilled water, we have dissociation or separation of the solution into component parts. In such a solution we shall have H-ions, OH-ions and undissociated molecules of the water, and H-ions, Cl-ions and undissociated molecules of the acid. The number of free H-ions is greatly increased because of the large number in the drop of acid, and the number of OH-ions is decreased because changes that occurred upon the addition of the acid to the water caused many of these OH-ions to unite with the H-ions to form molecules of water, thus losing their electric charge. Upon further addition of the acid the H-ions increase and the OH-ions decrease in number and we get a stronger acid. If a base were added to distilled water the conditions would be reversed and there would follow an increase of OH-ions and a decrease of H-ions.

Either the H-ion concentration or the OH ion concentration may be used as an index of the true condition of a solution, but it is customary to use the H-ion concentration. The hydrogen ion concentration is inversely proportional to the dissociation of the base: for example, a strong solution of sodium hydroxide has a very small H-ion concentration and a large OH-ion concentration; but its H-ion concentration can still be measured and used as an index. The different acids and also the alkalies vary in the degree of dissociation or ionization and therein lies the strength or weakness of the acid or alkali. Acids and bases dissociate to a greater extent than water, and, in turn, the strong inorganic acids like hydrochloric ionize or dissociate to a greater extent than do the weaker acids like acetic and other organic acids. We find that a one per cent. solution of hydrochloric acid is much more sour

than an equivalent solution of acetic, because a greater proportion of the hydrochloric is dissociated into H-ions. In other words, the H-ion concentration of an acid increases directly with dissociation; hence, if different acids be dissolved in the same ratio to water the H-ion concen-

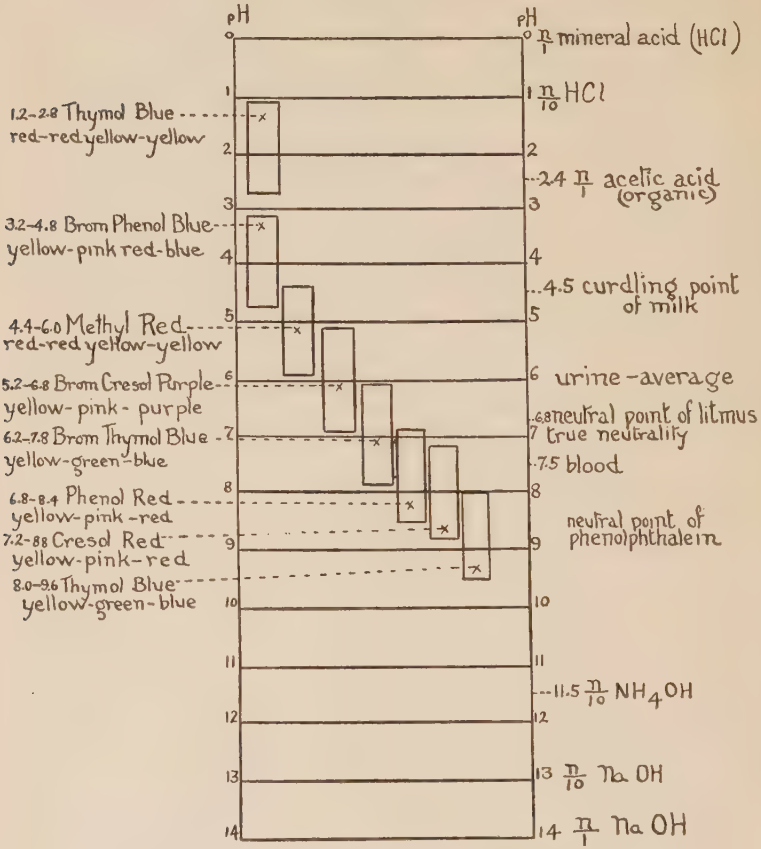

Fig. 144.—Color ranges in various H-ion indicators.

tration will be an index of the strength of each acid. Similarly, if different bases be dissolved in water in the same ratio the OH-ion concentration of each will be the index of the strength of each base.

For an explanation: Let us consider what we mean by the term "normal solution." In chemistry we are taught that a "quantity" normal solution of an acid is one that contains one gram of replaceable

hydrogen in one liter of water; a tenth normal is one that contains one-tenth of a gram of replaceable hydrogen, etc. Again, a quantity normal solution of any alkali is one that contains per liter an amount of OH chemically equivalent to one gram of hydrogen or actually seventeen grams by weight; therefore, a quantity normal solution of NaOH would contain 17 grams of OH (O equals 16, H equals 1). The above statements hold true for any quantity normal solution. The acidity or reaction of such solutions varies greatly with individual acids or alkalies. We know that the strength of an acid solution is determined not by the weight of replaceable hydrogen present, as it is in a quantity normal solution, but by the weight of dissociated electrically charged H-ions present in that solution and that the activity of a base depends on the weight of dissociated electrically charged OH-ions present. In order to provide a nomenclature to meet this situation, a solution is called "normal acid in reaction" when in a liter of such a solution there is one gram of H-ions and "normal alkaline in reaction" when it contains OH-ions equivalent to one gram of H-ions. The question of whether a "quantity normal solution" of any given acid is "normal acid in reaction" is determined by the dissociation of that acid. If, for example, the dissociation of a certain acid is one gram per liter when the solution is a quantity normal solution, then the quantity normal solution is also a normal acid solution in reaction. If, on the other hand, we have a quantity normal solution of another acid and we find that the dissociation of this acid is only a tenth, then the acid is only $^{n}/_{10}$ as far as reaction is concerned, even though it is a quantity normal solution. Compare $^{n}/_{1}$ acetic acid with $^{n}/_{10}$ HCl on Chart No. 1, and Chart No. 2. Thus we see that normal acidities, or better, reaction normalities depend upon the H-ion concentration and are expressed in terms of it.

The H-ion concentration of any solution depends on varying conditions of temperature, dilution, buffers, etc. "A buffer is any substance in a solution, which, because of its affinity for either acid or alkali, tends to preserve the original H-ion concentration, by combining with any acid or alkali which may be added, thus nullifying their effect."* Peptones, meat infusions, sodium borate, and many other substances often present in media act as buffers. When buffers are present the H-ion concentration or free acid of any solution will obviously be lower than it would have been with the same amount of acid added, if no buffer were in the solution. The actual acidity or reaction of such a solution would be measured by its H-ion concentration, while the total amount of acid present, free and combined in the buffers, would

*Park & Williams: *Pathogenic Micro-organisms*

be found by titration against an alkali using an indicator to determine end point.

The usefulness of a medium depends upon its actual acidity, *i.e.*, H-ion concentration. Because we use the H-ion concentration as a measure of actual acidity, it became necessary to find a convenient method of expressing this measure.

The actual numbers are too unwieldy to use in routine work. Even in pure water, where the H-ion concentration is 0.0000001 gram per liter, the figures are troublesome, and in strong alkaline solutions they become impossible to handle.

Sorensen suggested the use of logarithms—a "log" being merely an exponent which, when given to a number, called the base, raises that number to the required value. Study the following table which expresses the same thing in five different ways:

Substance	Concentration of H-ions in grams per Liter			pH
	Decimal	Fraction	In powers of 10	
$\frac{N}{10}$ HCl.	0.1	$\frac{1}{10}$	1×10^{-1}	1
Gooseberries	0.001	$\frac{1}{1000}$	1×10^{-3}	3
Pumpkin	0.000,01	$\frac{1}{100,000}$	1×10^{-5}	5
Pure water	0.000,000,1	$\frac{1}{10,000,000}$	1×10^{-7}	7
$\frac{N}{10}$ NaOH	0.000,000,000,000,1	$\frac{1}{10,000,000,000,000}$	1×10^{-13}	13

The word log is dropped, together with the minus sign (meaning a fraction or decimal) and for convenience, the arbitrary term pH, as suggested by Sorensen is used instead.

It will be noticed that where the grams of H-ion per liter are multiples of 10 the pH values are identical with the negative powers of ten. The differences in actual acidity are greater than they first appear —the acidity expressed by pH3 is one hundred times greater than that expressed by pH5 and that expressed by pH5 is one hundred times greater than that expressed by pH7. As the pH changes by one the acidity changes by ten, but in the opposite direction. Thus the pH is an invert sign, *i.e.*, its exponent decreases with increasing H-ion concentration. We may, therefore, say that acids have a value less than 7 and bases greater than 7, or, to speak in terms of H-ion

concentration—acids have H-ions in greater amount than 0.0000001 grams per liter and bases in less amounts. It is also possible to compute the OH concentration for any solution of which the pH is known, because there is a constant relation between them as expressed by the equation

pH plus pOH equals 14

The pOH may be determined for any solution by subtracting the pH value from 14; for example, an acid having a pH of 4 would have a pOH of 10. See Chart on p. 266.

The hydrogen ion concentration of a solution or its reaction may be determined in two ways, electrometrically or colorimetrically. The electrometric measurement of the H-ion concentration depends upon the fact that when a metal is in contact with an aqueous solution containing dissociated ions of that metal, there is an electro-motive force set up between the metal and the solution, which varies according to the concentration of the ions of this metal in the solution. The electro-motive force can be measured, and, by means of the proper formula, the ionic concentration of the metal in solution can be determined directly. Hydrogen, as we know, acts like a metal in this respect when brought into electrolytic contact with an aqueous solution containing H-ions, such as the medium to be tested.

The colorimetric measurements depend upon the color changes produced in certain indicators by so-called standard solutions of known pH values as previously determined electrometrically. Indicators are complex chemical substances, each one having its own characteristic but narrow zone of H-ion concentration within which its color changes and every shade of the color corresponds to a definite pH value. The indicators that have been used longest by the bacteriologist are litmus and phenolphthalein. Litmus has very little value in accurate pH measurements because of its uncertain chemical composition. Phenolphthalein gives accurate results within the pH limits between which its color changes (8.2 or 8.3 — 9.6) but its range is considerably on the alkaline side of true neutrality which is 7. Bacteriological work has more special need of H-ion determinations near neutrality or in the acid range. The indicators we use now are specially chosen because their colors are brilliant and are not reduced by bacterial action. Of special interest are the three whose pH ranges are nearest true neutrality: these are brom cresol purple with useful pH 5.2 to 6.8 with a color change from yellow through pink to purple; brom thymol blue whose useful pH is from 6.2 to 7.6 and a change of yellow through green to blue, being grass green at pH7; and phenol red with a useful pH range of 6.8 to 8.4 and a color change of yellow through pink to red.

Hydrogen-ion Concentrations

pH values	Reaction Normalities	Con. of Hydrogen or gms. H-ions per Liter
	Acid	
0.0	$\frac{N}{1}$	1
1.0	$\frac{N}{10}$	$\frac{1}{10}$
2.0	$\frac{N}{100}$	$\frac{1}{100}$
3.0	$\frac{N}{1000}$	$\frac{1}{1000}$
4.0	$\frac{N}{10,000}$	$\frac{1}{10,000}$
4.5	color change methyl orange	$\frac{3.2}{100,000}$
5.0	$\frac{N}{100,000}$	$\frac{1}{100,000}$
6.0	$\frac{N}{1,000,000}$	$\frac{1}{1,000,000}$
6.8	color change litmus	$\frac{1.6}{10,000,000}$
7.0	$\frac{N}{10,000,000}$	$\frac{1}{10,000,000}$
7.5	blood	$\frac{3.2}{100,000,000}$
8.0	$\frac{N}{1,000,000}$	$\frac{1}{100,000,000}$
8.5	color change phenolphthalein	$\frac{3.2}{1,000,000,000}$
9.0	$\frac{N}{100,000}$	$\frac{1}{1,000,000,000}$
10.0	$\frac{N}{10,000}$	$\frac{1}{10,000,000,000}$
11.0	$\frac{N}{1000}$	$\frac{1}{100,000,000,000}$
12.0	$\frac{N}{100}$	$\frac{1}{1,000,000,000,000}$
13.0	$\frac{N}{10}$	$\frac{1}{10,000,000,000,000}$
14.0	$\frac{N}{1}$	$\frac{1}{100,000,000,000,000}$
	Alkaline	

Acid to Neutral to From Alkaline

Chart of A. L. Wachter (modified slightly) showing H-ion concentrations.

A rough idea can be obtained of the H-ion concentration of any solution by simply finding which indicators give their acid color in it and which give their alkaline color.

*Indicator	Full acid color	Full alkaline color	Useful pH range
Thymol Blue	red (r. y.)	yellow	1.2—2.8
Brom Phenol Blue	yellow	blue	3.0—4.6
Methyl Red	red (r. y.)	yellow	4.4—6.0
Brom Cresol Purple	yellow (pink)	purple	5.2—6.8
Brom Thymol Blue	yellow (green)	blue	6.0—7.6
Phenol Red	yellow (pink)	red	6.8—8.4
Cresol Red	yellow (pink)	red (purple)	7.2—8.8
Thymol Blue	yellow (green)	blue	8.0—9.6

*See color chart, Fig. 144.

Accuracy, however, can be obtained only by actual comparison with the colors produced in the indicators by solutions of known H-ion

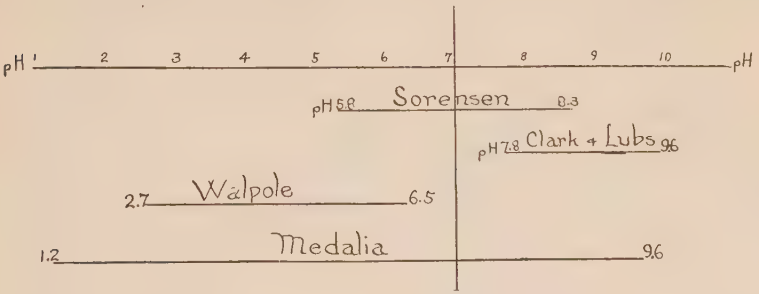

FIG. 145.—The ranges covered by various H-ion standard sets.

concentration. For this comparison several sets of standards have been devised: Sorensen devised a set whose useful range is pH 5.8—8.3; Clark and Lubs devised another set ranging from 7.8—10. Walpole devised another set all in the acid range from 2.7—6.8.

These solutions require too accurate work and detailed care for ordinary laboratory use, and each also covers a relatively narrow range. From the original Barnett and Chapman method, Medalia devised a much simpler one which yields results accurate enough for practical purposes and which has a wider range of pH values—from 1.2 to 9.6.

The Medalia set comprises two series of test tubes—one series of dilute acid and the other of dilute alkali. Into the first acid tube the largest amount of an indicator is put and into the first alkaline tube the smallest amount of that same indicator and these form a pair and are viewed together in transmitted light. Into the next acid tube is put the

next largest amount of the same indicator and into the second alkaline tube the next smallest amount and so on. The sum of the amounts of indicator in every pair is always the same.

Looking at the light through both tubes of any pair the same color is obtained as when a solution of the proper H-ion concentration is viewed

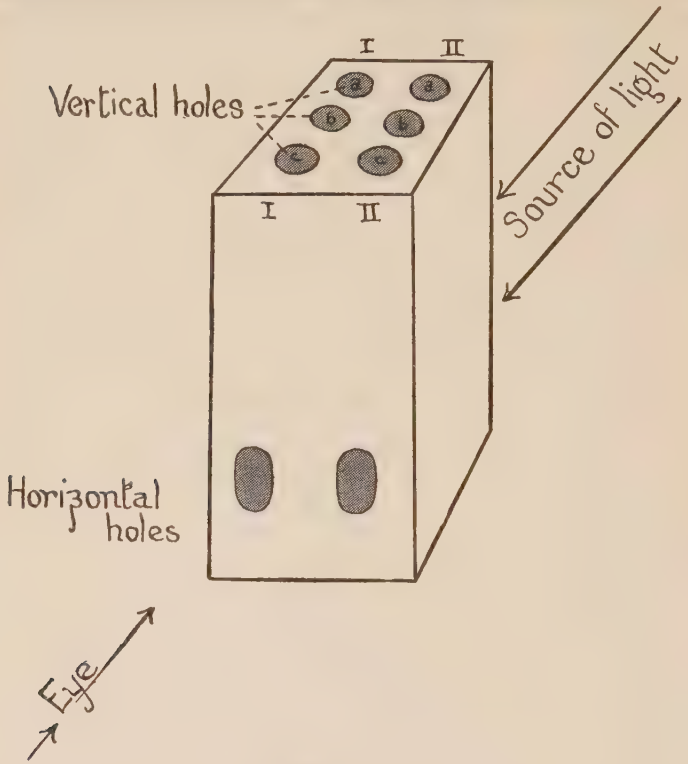

FIG. 146.—A comparator box or block.

In vertical holes, (a) and (b), No. I, put an acid and an alkali tube of same pH containing the indicator; in hole (c) put the tube of material to be tested.

In row No. II, put two tubes of distilled water; in (c) place the tube of material to be tested, containing the same amount of indicator as is in both of the acid and alkaline tubes of known pH value.

With the eye at the horizontal holes, match the color seen in row II with any pair of indicator tubes containing the same indicator. When the two colors seen (I and II) match we have the H-ion concentration of the material being tested.

with an amount of the indicator equal to the sum of the amounts in the two tubes of the standard. For rough work it is often possible to determine the pH value of the solution to be investigated by merely holding the test tubes in the hand between the eye and the source of

light. For accurate work a comparator box is necessary. A comparator box is a block of wood with six vertical holes in two rows of 3—each hole made so that the tube will fit snugly, and two horizontal holes or slits bored through the block, one cutting through each row of vertical holes. (See Fig. 146.)

The practical use or value of using indicators to determine acidity combined or free is threefold:

1. To determine the reaction of media in order to adjust it during the process of preparation.
2. To determine end reaction of media after bacterial growth.
3. To be incorporated in culture media to indicate changes in reaction due to growing bacteria.

Besides these important laboratory applications, the relation of acidity to commercial processes, *e.g.*, the effect upon the processing time necessary in canning, opens up a wide field for the practical application of color indicators.

GLOSSARY

Note.—These definitions are not meant to be complete technical definitions, but to aid a beginner in using this outline text.

Aërate. To expose to the action of air; to force air through.

Aërobic. Growing in the presence of air or oxygen. Demanding air (free oxygen) for normal growth.

Agar. The term agar is used both for a substance expressed from seaweed and for a combination of that substance and meat broth into a solid medium for cultivating bacteria. It forms a semi-transparent substance resembling gelatin in appearance.

Agar Plate. A double glass dish (Petri dish) containing a layer of agar which furnishes food for the growth of bacteria (Fig 85).

Agglutinins. Substances accumulating in the blood as a reaction to bacterial growth or action; these agglutinins cause the sticking together or agglutination of the related bacteria (typhoid bacteria by typhoid blood), often a preparatory step in their destruction by the white corpuscles.

Algæ. Plants of relatively simple structure, commonly growing in water. Common illustrations are the slimy green scum on ponds and the stringy green masses on dock piles. Bacteria are usually classed in the lowest division of the algæ, although bacteria do not possess the green coloring matter characteristic of most algæ.

Alkali. A compound of hydrogen and oxygen with such substances as sodium or ammonium; these compounds are very soluble in water and can neutralize acids. Lime and other compounds that have such neutralizing power for acids are popularly spoken of as alkalies.

Allergy. A name given to the milder evidences of protein sensitiveness sometimes shown by human beings. (See Anaphylaxis.)

Anaërobic. Not demanding free oxygen for normal growth (see Fig. 77). Organisms which cannot develop in the presence of oxygen are spoken of as "strict" or "obligate" anaërobes.

Anaphylaxis. (Without protection.) A term covering various forms of protein sensitiveness, from itching of the nose or hives to complete collapse and death. Such sensitiveness commonly develops only after an initial injection or absorption of a given protein (pollen, serum). Severe anaphylactic reactions are not common in man.

Anaphylactic. Symptoms include difficulty in breathing, convulsions and even death; itching, "hives" and such minor symptoms are found in allergy.

Antibodies. Reacting substances formed by the body and accumulating in the blood in response to irritation such as bacterial action. These include antitoxins, agglutinins, opsonins, and lysins as discussed in this text.

Antigen. A substance which arouses specific antibodies or which unites with them in tests, such as blood or serum tests for whooping cough. A vaccine is really an antigen.

Antiserum. Blood serum from an animal immune to a given disease, and, therefore, containing one or more kinds of antibodies (antitoxins, agglutinins).

Antiseptic. Against or preventing sepsis (or decay). In treating wounds it is not always sufficient to make sure that the dressings are aseptic. The wound itself may have in it injurious bacteria which came from the air, the near-by surface of the body, or which got in at the time of injury. Sterile or aseptic dressings will not prevent injurious organisms from developing. To hinder their development, chemicals are sometimes used either on the dressings or applied directly to the wounds. Since these prevent sepsis, they are called antiseptics. Care must be taken that antiseptics are not too strong, or they will destroy the body cells, or at least irritate them and so delay healing. (See Aseptic.)

Antitoxins. The substances or antibodies formed in the body in response to the irritating or poisonous toxins of such bacteria as diphtheria and lockjaw are called antitoxins. These antitoxins neutralize the toxins, but do not aid the white corpuscles nor themselves destroy the bacteria. We are most familiar with the word antitoxin in connection with the formation of such substances in large quantities by the horse, which enables us to use the blood of such horses to protect human beings (diphtheria antitoxins).

Arthritis. A disease of the joints with symptoms resembling rheumatism or gout.

Aseptic. Aseptic means without decay or sepsis. (See Septic.) Wounds should be kept in an aseptic condition and substances in contact with wounds should be aseptic, that is, without any decaying particles or any organisms that could cause sepsis. All sterile substances are aseptic. Dressings and instruments may be made aseptic by boiling, by subjecting them to hot steam, or by such chemicals as alcohol and chloroform. If other chemicals which do not evaporate, such as carbolic acid or corrosive sublimate, are used for dressings, they are made antiseptic rather than merely aseptic. (See Antiseptic.)

Asexual Reproduction. Cell division in ordinary growth, budding (yeasts), and spore formation bacteria) are all three asexual methods of reproduction; fusion of cells or their nuclei does not occur in this type. (See Sexual Reproduction.)

Auto-intoxication. The digestion or breaking down of protein foods includes many temporary or transitional stages, some of which are poisonous. Nervous or other functional derangements of the digestive processes may lead to the accumulation and absorption of unusual amounts of intermediate poisonous stages; this self-poisoning is termed auto-intoxication. Even in health, the organisms present in the alimentary canal may vary greatly, and so cause temporary difficulties (indigestion, "auto-intoxication"). (See Food Poisoning, also.)

Autopsy. An examination of a dead body to determine the cause of death, the seat of the disease, etc.; a post-mortem examination.

Bacillus (pl. Bacilli). A rod-shaped bacterium; term usually used for the forms possessing motility or the power to form spores (Fig. 72, II).

Bactericidal. Able to kill bacteria; carbolic acid, alcohol, etc., are bactericidal substances.

Bacterin. (See Vaccine.) Bacterin is sometimes used as a name for killed cultures of bacteria which are given to produce immunity to a given disease. The vaccine used in typhoid prevention is technically a bacterin.

Bacterium (pl. Bacteria). A rod-shaped micro-organism, differing from bacilli in most classifications in not forming spores. Bacterium or bacteria is used as a general descriptive term for any of the related groups of cocci, bacteria or bacilli.

Blood Count. A count of the total number and proportion of white and red corpuscles. This count is made by putting a carefully measured amount of accurately diluted blood on a special slide, which holds a given amount of blood over an area marked off in tiny squares to facilitate counting. The count is made through a microscope.

Botulism. Food poisoning due to the formation of toxin by *B. botulinus*, usually in meats or vegetables which have been insufficiently heated (cooking, canning).

Canada Balsam. A resinous transparent liquid obtained from the balsam (or fir tree) which is useful for permanent microscopic mounts, as it has about the same refractive index as glass.

Carrier. A person who, though apparently well, carries (throat, intestines) the organisms of disease. In such people the reactions are sufficient to partially protect the individual, but not sufficient to destroy all the invading organisms.

C.C. (See Cubic Centimeter.)

Cedar Oil. An oil obtained from the Cedrella tree. Used for temporary mounts and examination of microscopic objects, because, like Canada Balsam, its refractive index is very near that of glass.

Centigrade. The centigrade thermometer has but one hundred divisions or grades (therefore, literally centigrade) between freezing and boiling. See Fig. 70 for corresponding centigrade and Fahrenheit temperatures with rules for translating one scale into the other.

Centrifuge. The principal part of a centrifuge is a horizontal disk or wheel which can be revolved very rapidly—by hand power or machinery. In this disk are little cavities in which tubes or flasks of liquids (bacterial cultures, water containing bacteria) may be suspended. As the speed of the disk increases, these tubes tend to take a position approaching the horizontal, and the heavier substances in these liquids (bacteria, dust particles), thrown outward by centrifugal force, collect in the ends of the tubes. The top or lighter part of the tube contents can then be poured off, and the heavier part (bacteria) retained relatively free from the other substances. (Fig. 87, II.)

Certified Milk. Milk produced under regulations issued by a medical commission, and covering usually the health of the cow, milking conditions and the number of bacteria at the time of delivery.

Chlorophyll. The characteristic green pigment of plants (leaves) which enables them to make food (sugars) from carbon dioxide.

CO_2. Carbon dioxide.

Coccus (pl. Cocci). A globular bacterium. The genus Diplococcus includes such organisms which characteristically grow in pairs; the genus Streptococcus those which grow in chains.

Colonies. (See Fig. 67.)

Communicable. Diseases readily transferred from one person to another are now spoken of as communicable. This term is supplanting the older term contagious.

Cretaceous. Lime-like.

Cubic Centimeter (c.c.). A small unit of size in the metric system, now commonly used in scientific measurements and descriptions. A cubic centimeter of water weighs 1 gram; it can be visualized better by remembering that a cubic centimeter of such liquids as water contains about 16 drops. The word mil (*mille*, thousand) is sometimes used for one cubic centimeter, as it is one thousandth of a liter, the larger liquid unit of the metric system (a little over 1 quart).

Denitrification. The breaking down of proteins or nitrogen-containing compounds: proteins to amino-acids, nitrates to nitrogen, etc.

Diagnosis. A conclusion based upon a careful study of existing conditions and the characteristic signs or symptoms thus observed. A diagnosis was formerly based upon clinical symptoms only; at present chemical, serological and microscopic examinations of blood, pathological material, etc., are often made to confirm the clinical diagnosis.

Dialysis. The passing of soluble substances through membranes: natural plant or animal cell membranes, or artificial membranes, such as dialyzing bags. (See Osmosis.)

Diastase. An enzyme which changes starch to sugar (or sugar to starch).

Disinfect. To render free from infection or disease-producing organisms; this does not mean that after disinfection no microorganisms are left, but that no disease-producing organisms have survived. Usually, however, there are few or no bacteria in disinfected materials or substances. (See Sterilize.)

Distilled. Water is distilled by boiling it and collecting and cooling the steam or vapor, thus condensing it back to water. This distilled water does not contain the minerals and organic materials present in the original water. If collected and cooled in sterile vessels, it may be *sterile* water as well, though distilled water is not necessarily sterile water, as spores may possibly be carried over in the steam, especially from rapidly boiling water.

Effluent. That which flows forth or out, *e.g.*, the water flowing out from the bottom of a sewage filter.

Endemic. Applied to any disease produced and propagated by local conditions; *e.g.*, malaria and hookworm are endemic in certain areas of the United States.

Enzymes. Substances produced by living cells which bring about definite changes in those cells, their food materials, or their stored substances, *e.g.*, diastase, enzymes change starch to sugar.

Epidemic. (Literally, upon the people.) Applied to sudden and widespread manifestations of disease, *e.g.*, the recent influenza epidemic. Endemic diseases may assume epidemic proportions.

Eustachian Tube. A tube connecting the cavity between the nose and throat with the ear. Infection from the mouth, etc., very easily spreads into this tube and so into the ear, often penetrating the spongy (mastoid) bone near the ear (mastoid abscess).

Exudate. The more or less liquid material found in a lesion or exuding from an inflamed area (drip from nostrils or mucus in nasal passages in a "cold in the head").

Feces. The discharges from the intestines or bowels.

Fibrin. A protein substance in the blood which helps form the "clot" in coagulating blood. Defibrinated blood is called serum, the corpuscles being held in the "clot."

Filterable. Able to pass through a standard filter, such as a porcelain filter.

Fimbriate. With a finely-divided or fringed margin.

Flora. The plants present in a given region. A list of the bacteria present in the intestine may be termed the "intestinal flora."

Focus (pl. Foci). A center; the site or seat of infection (tonsils in diphtheria).

Fomites. Substances capable of absorbing, holding, and transporting infectious micro-organisms; mucus, clothing, etc., come under this heading.

Food Poisoning. This is a general term applied to all stomach or intestinal disturbances due to foods. It covers conditions caused by foods naturally poisonous (*e.g.*, certain mushrooms), by introduced chemicals (*e.g.*, preservatives), by toxins found in the food before it is eaten (as in botulism), or more rarely still, by ptomaines; usually, food poisoning is due to the activity of organisms (dysentery, paratyphoid) after they reach the intestines.

Food Sensitives. Individuals who experience more or less discomfort ("hives," itching, "running" of the eyes or nose) following the eating of one or more specific food substances such as milk, shellfish, eggs.

Germicidal. Used for substances or agents that can kill germs or micro-organisms. Acids, high temperatures, etc., have germicidal power. Germicidal substances are also called disinfectants.

Glycogen. A starchy substance commonly called "animal starch," which is formed in the liver. As needed, it is changed back to sugar and distributed by the blood. Glycogen granules have been demonstrated in yeasts.

Gonorrhœa. A dangerous disease affecting such delicate membranes as the eyes and the genital area. It is sometimes transferred through soiled towels, bedding, and personal articles, as well as by personal contact.

Gram. A unit of weight. One cubic centimeter of pure water at its greatest density (4° C., or 39° F.) weighs one gram. Gram and cubic centimeter are, therefore, practically interchangeable for water, but not for very light, heavy, or dry substances.

Hæmoglobin. The compound responsible for the coloring matter of red corpuscles and important as a carrier of oxygen. Color tests to determine the per cent. of hæmoglobin are often important in diagnosis.

Hæmolysis. The "dissolving" or separation of the red coloring matter from the red blood cells.

Hay Fever. Supersensitiveness to pollen or other air-carried particles. Unusual permeability of the (nasal) membranes is probably a related condition and may explain the erratic manifestations of such affections.

H-ion. A term used in discussing acidity. (See Appendix.)

Hookworm. Small round worms which cause the hookworm disease.

Host. An organism that harbors another as a parasite. In typhoid or hookworm infections, man acts as host for the typhoid or hookworm organisms.

Hydrolysis. Hydrolysis is a chemical change attended by or dependent upon the presence of water. Many enzyme changes are of this kind; for example, when starch is changed to sugar the addition of water is the first step.

Immune. (See Immunity.)

Immune Blood. Blood from a person or animal immune to a given disease; blood taken after recovery from vaccination or an ordinary attack of the disease.

Immunity. This is defined as freedom or exemption from; the literal meaning, "not in service to," carries the same implication.

Incubation. When bacteria are kept at temperatures favorable for growth or multiplication, they are said to be incubated. We also speak of the time period when bacteria are multiplying in the body as the incubation period; this extends from the time when the disease organism enters the body to the time the patient "comes down" with the disease.

Inert. Neutral; devoid of active chemical properties.

Infection. Disease organisms that multiply in the body cause infection. Infection may be localized, as in a boil, or general, as in blood poisoning.

Infectious. Caused by disease-producing organisms, or capable of producing disease through the transfer of such organisms.

Inhibited. Prevented from growing or multiplying in number. Chemicals which sterilize or disinfect in strong solutions may only inhibit bacterial growth or action when used in weaker solutions.

Inoculate. To introduce directly into the body, as into the skin, the abdominal cavity, or a blood-vessel; used mainly for the introduction of micro-organisms; curative substances (serum, etc.) are more commonly spoken of as *injected.*

Inorganic. Not now or ever having been a living organism; mineral rather than organic is an easily understood difference, though organic substances contain minerals. (See Organic.)

Involution Forms. Abnormalities (size, shape) formed by cells with age, undue stimulation (foods, wastes), etc.

Lactic. Commonly applied to milk changes, such as lactic fermentation. The lactic acid produced in souring milk is common in many other fermenting substances containing sugar.

Leaching. Separating or washing out soluble matter by draining or percolating.

Lesions. Injuries, diseased areas, or morbid changes in organs or tissues; these include bruises, ulcers, and inflamed as well as disintegrated areas.

Leucocytes. (See White Corpuscles.)

Liter. A metric system unit of volume which contains 1000 cubic centimeters. (A quart is about 960 cubic centimeters.)

Litmus. A plant extract giving different color reactions with acids (red) and alkalies (blue).

Lymph-nodes. Small lymph-glands or collections of lymph tissue. Certain types of white corpuscles are produced in these lymph-glands.

Lymphocytes. (See White Corpuscles.)

Lysins. Substances (antibodies) formed in the body as a reaction to bacteria or other foreign cells, and having the power to dissolve them.

Medium (pl. Mediums or Media). Substances used for the cultivation or growth of micro-organisms; milk, beef broth, gelatin, are common media. Special media are used for certain organisms; these may contain egg (tuberculosis), serum (diphtheria), special sugars, etc.

Meninges. The enveloping membranes of the brain and spinal cord.

Metabolic. (See Metabolism.)

Metabolism. A comprehensive term for all the cell changes, including the change of stable non-living food substances to complex unstable living material and the breaking down of that living material to simpler and more stable substances; metabolism includes all the upbuilding and energy-producing changes in a cell.

Metric System. A system of weights and measures commonly used in Europe, and now generally adopted in this country for scientific work. It is much simpler than our own systems, and has small units more suitable for fine measurements. The metric units used in this text are described in this glossary under millimeter, cubic centimeter and gram. The centigrade thermometer is based on the same unit system (varying by tens or hundreds).

Micron. (See Millimeter.)

Micro-organism. An organism of very small size, making a microscope necessary for its detailed study.

Mil. (See Cubic Centimeter.)

Milligram. One-thousandth of a gram (*mille*, one thousand).

Millimeter. One-thousandth part of a meter; about $^1/_{25}$ of an inch. The millimeter is further subdivided into thousandths or micro-millimeters (microns). See p. 92.

Mitosis. Cell division characterized by complex changes (Fig. 35) as contrasted with simple constriction of a cell and its nucleus into two new cells.

Mordant. See p. 115.

Motility. The power of locomotion.

Mucus. The viscid secretion of certain membranes; we are most familiar with it as the excessive secretion of those of the nose and throat attending colds.

Mycelioid. Mold-like; thready in texture, like a mycelium.

Mycelium (pl. Myceliums or Mycelia). The thready vegetative growth characteristic of most molds.

Nitrification. The process of building nitrates from ammonia. Two types of bacterial activity are included: Ammonia to nitrites (Nitrosomonas) and nitrites to nitrates (Nitrobacter).

Nitrogen Fixation. The utilization of free atmospheric nitrogen to form nitrates (Rhizobium).

Non-pathogenic. Not capable of causing or producing disease.

Opsonins. Antibodies produced as a reaction to invading organisms which aid the white corpuscles in digesting and destroying them.

Optimum. Used to designate the condition or conditions producing the best results.

Organic. All organic substances contain carbon as an essential element; usually applied to substances which are or have been part of living organisms; flour, milk, and spores are organic substances in contrast to iron rust, table salt, and air, which are inorganic. (See Inorganic.)

Osmosis. The term osmosis is now often limited to the passage of water through membranes, dialysis being used for the passage of substances in solution, such as sugars, salts, and acids. There is a constant exchange of substances in and out of cells by these two processes.

Oxidation. The union or combination of oxygen with other substances. Oxidation breaks down or decomposes many complex substances; this change in structure from complex to simpler forms liberates energy. Much of our energy (and heat) is obtained by oxidizing food substances, hence the need for a rich blood supply of oxygen. Foods are sometimes split into simpler substances without oxygen entering into the change; oxidation is usually, however, the more economical way of producing cell energy.

Parasite. An organism which lives upon another living organism or host. Tapeworms, bacteria, and molds which cause ringworm are examples of human parasites.

Paratyphoid. An intestinal disease, or the organism producing it; it resembles typhoid, therefore the prefix, *para.*

Pasteurization. A method (introduced by Pasteur) of heating substances (wine) to destroy undesirable or spoilage organisms. As applied to milk, pasteurization means heating the milk to a temperature which insures the killing of all pathogenic organisms which might occur in contaminated milk.

Pathogenic. Capable of causing or producing disease.

Pathogens. Used to designate micro-organisms which are capable of causing or producing disease.

Petri Dish. A double glass dish or plate (Fig. 85), used to cultivate micro-organisms. (See also Agar Plate.)

Phagocytes. (See White Corpuscles.)

Phenolphthalein. A colorless organic substance giving a reddish reaction in alkaline solutions. (See Appendix.)

Photosynthesis. The building up or synthesis of sugar by green plant cells from carbon dioxide and water; light is essential for this process.

Plasma. The liquid part of the blood, in contrast to the cellular parts: red and white corpuscles.

Plasmolysis. A shrinkage or collapse of the cell attendant upon the loss of water. The "keeping power" of brines and strong sugar solutions is due to their power to abstract water from micro-organisms, and thus prevent them from growing.

Plate Counts. Plate counts are made to determine the number of bacteria in a given amount of milk, water, etc. (Fig. 107.)

Poliomyelitis. Infantile paralysis.

Polluted. Containing organic material, the products of plant or animal decay.

Precipitate. To cause to fall or settle more rapidly than would otherwise occur.

Precipitins. Substances found in the blood as a reaction to foreign bodies in the blood stream, such as bacteria and the blood-cells of another animal; when mixed with solutions of the related cells precipitates appear. (See Fig. 79, II.)

Presumptive Tests. Because of the difficulty in identifying bacteria by their shape and size, we depend partly on their products or results when grown in certain food materials. For example, the colon bacterium, unlike most bacteria, can use lactose, forming from it acid and gas with a definite proportion of hydrogen and carbon dioxide. If water or milk containing colon organisms is added to lactose broth, we get much the same results. Knowing this, we use lactose as a test substance in examining water, milk, etc. If the results are like those obtained with a culture of colon organisms, we conclude or presume that *coli* is present. Such

tests are therefore called presumptive tests. (See p. 98.)

Protein Sensitives. Food sensitives and "hay fever" subjects are supersensitive to special proteins (eggs, pollen) which apparently enter the blood stream through membranes in the associated areas (intestinal wall, nasal membranes). Such sensitiveness to foreign protein (typhoid proteins in typhoid vaccination) is the basis of all our reactions against disease. The first meaning is the one usually meant in popular use of the term, however.

Protoplasm. The foundation substance of all living cells. Variously differentiated forms of protoplasm are found in the nucleus, cytoplasm, etc. (Formerly sometimes used for cytoplasm.)

Protozoa. The lowest group of animals; simple one-celled forms. (Figs. 38 and 54.)

Ptomaines. These are alkaloid-like substances, formed in the decomposition of proteins (amino-acids). Some of them are poisonous, though most are not. (See Food Poisoning.)

Pulmonary. Relating to the lungs, as pulmonary tuberculosis.

Putrefaction. Decay or decomposition, especially the types accompanied by the formation of objectionable odors; these are very commonly due to organisms which do not get their energy by oxidation.

Refractive Index. Substances which bend or refract light rays to the same degree are said to have the same refractive index. Cedar oil and Canada balsam have approximately the same refractive index as glass. (See Fig. 6 in this connection.)

Respiration. Respiration is essentially a cell process, another name for the oxidation processes of the living body. Breathing, or inhaling and exhaling air, is an accessory to respiration, but is not respiration itself—as the term is now generally used in this chemical sense.

Rickets. A nutritional disease of children, characterized by malformation of the skeleton and lack of muscular control or co-ordination.

Saprophyte. An organism which lives on organic material, but not on a living host. Bacteria or molds living on bread, dead fish, milk, are all saprophytes. Most bacteria are saprophytes.

Saturated (Saturation). When a liquid has dissolved or absorbed all it can of a given substance, it is said to be saturated, or to make a saturated solution. Saturated steam means steam having the largest possible amount of water present in it in vapor form.

Scurvy. Authorities differ as to the relative causal importance of diet and micro-organisms in this disease, which is characterized by anæmia, debility, bleeding gums, and scab-like manifestations on the skin.

Sepsis. (See Septic.)

Septic. Undergoing or showing signs of decay. A septic tank is one where sewage is held until it decays or decomposes past the ordinary malodorous stages. A septic wound has decay-producing organisms growing in it; pus, "proud flesh" are indications of such septic conditions.

Serology. Serology covers the tests and examinations of blood (serum) which deal with the presence (or absence) of antibodies and their relation to immunity and disease.

Serum (pl. Serums or Sera). The liquid of the blood (plasma) lacking not only the red and white corpuscles but the fibrin of the plasma as well. (See Whole Blood.)

Sexual Reproduction. Reproduction in which two cells or nuclei fuse together into one fertilized cell. Sexual reproduction is not seen in bacteria, but it occurs in the protozoa (Fig. 55) as well as in all the higher groups, where specialized egg and sperm cells form the fertilized egg cell from which the next plant or animal develops later.

Sludge. The semi-solid part of sewage left after crude sewage has been sedimented, or acted upon by bacteria.

Spirillum (pl. Spirilla). One of the classes of spiral-shaped bacteria is called *Spirillum; Vibrio* is another class of very short spirals. (See *Spirochæta.*)

Spirochæta. The protozoa also include a group of spiral-shaped organisms called *Spirochætæ.* Spirochætes cause such diseases as relapsing fever and syphilis. (Fig. 56.)

Spore. A stage in the life history or development of some bacteria. Spores are quite resistant to most unfavorable conditions, and are usually formed in response to such environmental influences.

Sputum. Mucus accumulating in or ejected from the mouth or throat.

Stains. Special dyes or stains are used to color bacteria so they may be seen more definitely. No real progress was made in identifying bacteria until after the discovery of aniline dyes by Perkin.

Sterile. Free from micro-organisms.

Sterilize. To free from all micro-organisms by such agents as heat or chemicals. A stronger term than disinfect, which insures the killing of only disease-producing organisms.

Streptococci. Coccus or globular organisms, which adhere in chains. A rather short-chained type is shown in Fig. 108.

Symbiosis. Living in combination with profit to each organism, *e.g.*, nitrogen-fixing bacteria live symbiotically with clover, beans, etc.

Syphilis. A dangerous disease sometimes transferred by drinking-glasses, towels, bedding, or personal articles used in common, but mainly by personal contact.

Tetanus. Lockjaw organisms or the disease caused by them.

Therapeutic. Having curative or healing properties.

Toxins. Many bacteria form specific poisons or toxins. These toxins may be excreted freely and accumulate in the blood, irritating the body cells generally, as in diphtheria, or affecting definite tissues, as the nerve tissue in lockjaw (tetanus).

Trichinella (Trichina). Round worms (as opposed to flat types, like tapeworms) which live in pigs as well as man. They injure mainly by perforating the intestinal walls and invading the muscles.

Trypanosomes. Protozoa causing various diseases in animals. (Fig. 8, II.)

Tuberculin. Killed tuberculosis organisms, used mainly in testing hypersensitiveness (fever, local inflammation) to tuberculosis organisms; such hypersensitiveness is taken to indicate present or recent infection.

Vaccine. Vaccine is a term used for substances, such as bacteria, which are inoculated into the body to protect it against disease. Vaccines are made of either living or dead organisms or their products (toxins): living organisms in smallpox vaccine, dead ones in typhoid vaccine, and toxins in diphtheria vaccine. When living organisms are used, they are first weakened in some way to lessen their effect.

Vegetative Stage. Active growing stage as opposed to resting or spore stage.

Virulent. Highly pathogenic or extremely toxic or poisonous.

Virus. A term for any unknown causal agent of disease. The material used to vaccinate against smallpox is commonly spoken of as smallpox virus. Similarly we speak of the virus of hog-cholera, or of foot-and-mouth disease. When the causal organism is known, it is usually spoken of by its group or generic name (streptococcus, trypanosome, spirillum, etc.) or by such general names as bacterium or protozoan.

Vital Resistance. The resistance of living organisms against such untoward agents as bacteria and fatigue. Upon the individual's resistance depends his freedom from disease, or the severity of the attack. This resistance is in great part dependent upon the activity of the white corpuscles, and the production of such antibodies as antitoxins and opsonins. How much is due to the chemical condition of the cells themselves and to such substances as vitamines is not yet determined, though these are known to be important.

Vitamines. Substances present in various foods and known by their effects rather than chemical or other characteristics. In bacteriological media the presence or absence of a given vitamine affects growth or multipli-

cation. Nutritional disturbances and increased susceptibility to disease may result from their absence in human beings.

White Corpuscles. The blood contains two kinds of corpuscles, red and white. The white corpuscles vary more than the red in size and activity; although several kinds are found in all human blood, they may be divided into two main groups, those that contain but one nucleus (lymphocytes) and those that contain several nuclei (leucocytes). White corpuscles, though relatively few in number, are most important; they help in blood clotting, in keeping a protein balance in the blood, etc. Those possessing several nuclei have another important function: they surround or envelop and finally digest bacteria, thus protecting the body against invading organisms. Sometimes dozens of bacteria may be seen inside one white corpuscle.

Whole Blood. Literally whole blood, without subtracting the corpuscles. Blood less corpuscles is spoken of as plasma; if the fibrin is also taken out, it is called serum; in clotted blood the liquid serum collects at the sides or top of the jar or container, the corpuscles (red and white) and the fibrin forming the clot.

Zymase. An enzyme (yeasts) which can break down glucose ($C_6H_2O_6$) to carbon dioxide and alcohol.

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See also the special references given at the end of each chapter.

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SAMPLE EXAMINATIONS

(Designed for approximately two fifty-minute periods. See also review questions at the end of each chapter.)

I

1. (50 min.) Discuss the milk question from a bacteriological standpoint. Give first a topical outline showing the relation of the parts to the whole discussion.

2. (20 min.) a. Give the best set of rules you can frame to protect yourself from infection (wounds and diseases in general).

b. Suggest additional rules for others, aimed to insure for yourself further protection.

c. What gaps are still left? or what is necessary to secure the execution of (b)?

3. (30 min.) Define the following; select first ten demanding paragraph definitions, and define the rest in single sentences:

mycelium, spore, cytoplasm, denitrifying, protozoan, anaërobic, lactic acid fermentation, enzyme, respiration, photogenic, bacteria, intracellular enzymes, optimum temperature, ultra-violet disinfection, sterilize, virulent, antibody, nitrogen cycle, antitoxin, agglutinin, presumptive test, differential medium, sand filter, legume nodules, bactericidal, alcohol, beer, vinegar, disinfect, toxin, septic tank, vaccine, infection, immunity.

4. (Additional and optional.) Discuss the economic phases of bacteriology which deal with the controlled growth or action of microorganisms.

II

1. (70 min.) Make a tabulated outline of microbiology as it affects man (in its broadest sense); topics—not facts—are desired as final divisions of the outline.

Underline once (1) those you could use in teaching in high schools; or (2) twice those you consider essential to a housekeeper.

2. (30 min.) Discuss (1) the one most important to the U. S. as an agricultural country; (2) the one you feel most able to discuss fully.

III

1. (30 min.) a. Describe briefly the various methods of examining milk.
   b. the methods of securing a high grade milk supply or
   c. the regulations concerning the milk sold in New York City.

2. (10 min.) How is water examined? Include one presumptive test.

3. (25 min.) (a) Describe the nitrogen cycle; or (b) list the economic phases of microbiology, dealing with the inhibition of the growth and other activities of micro-organisms.

4. (10 min.) Give five rules of conduct based upon bacteriology that you feel are important for you.

5. (25 min.) Define or give the most important fact relating to the following: disinfectant, deodorizer, agglutinin, pasteurize, chlorophyll, objective, condenser, organic, canning, bacteria, protozoa, toxin, antitoxin, vaccine, differential medium, pathogenic, cheese, pour plate, aërobic, denitrifying, nitrifying, Pasteur, Koch, solid media, and either Semmelweis, Weigert, or Metchnikoff.

IV

1. (10 min.) List with brief descriptions or definitions all the evidences of life shown by bacteria.

2. (Answer either a or b; 20 min.) a. Diagram the nitrogen cycle, indicating the parts performed by bacteria.
   b. Make a similar cycle for some other element, such as carbon, oxygen, or potassium.

3. (5 min.) Name the best liquid (or gas) disinfectant for general use. Justify briefly your selection. How do disinfectants act? Give two ways in which their efficiency may be increased.

4. (15 min.) How would you treat the following?
   —a freshly cut finger,
   —an abscessed wound,
   —dishes used by a diphtheria suspect,
   —intestinal discharges of a typhoid patient,
   —ready-made clothing that is not washable,
   —a room previously occupied by a tuberculous person,
   —clothing with a scarlet fever history which you wish to give away.

5. (30 min.) List seven to ten facts or principles that you consider all important for a teacher (or housekeeper), and which would make for safety, if no others were followed or practised. Show how each would affect our daily routine.

6. (10 min.) Define

microbiology	nucleus
bacteria	toxin
protozoa	vaccine
plasmolysis	antitoxin
cell	antiseptic

7. (10 min.) Name ten important historical dates or facts in microbiology; or give four important contributions to the science and show their later influence on its development.

A KEY FOR SOME OF THE COMMON BACTERIA

This key is based upon the keys and tables in Bergey's *Manual of Determinative Bacteriology.* Most beginning students are wholly unfamiliar with the principles of classification, and a plan of this kind has been found helpful in emphasizing the essential likenesses, as well as the differences, upon which all systems of classification are based. The following tabulation is far from complete; several groups or *genera* given by Bergey are omitted entirely as well as most of the bacterial *species.* The aim has been to give in a related scheme—brief enough to be comprehended as a whole by the student unfamiliar with such work —some idea of the interrelationship of the various genera, and to enable the beginner to place in such a scheme some of the organisms with which he is more or less familiar, by activity or by name, *e.g.*, the typhoid bacterium, the hay bacillus and the nitrate organisms of the soil.

Abbreviated as it is, it is possible to trace to the genus such organisms as *coli, subtilis* or staphylococcus from the characteristics which the student could determine in the laboratory. At that point, if time allows, he should be encouraged to use the generic tables scattered throughout Bergey's Manual and trace the organism down to its specific name.

EXERCISES WITH THE KEY

1. From this key find at least five ways in which rods belonging to the true bacteria may differ from each other.

2. What characteristics have dysentery and typhoid bacteria in common? What like characteristics have *Bacillus subtilis* and *Lactobacillus viscosus? Proteus vulgaris* and the plague organism, *Pasteurella pestis?*

3. Why does the typhoid organism not belong in the genus *Bacillus?* Or Shiga's dysentery bacterium in the genus *Lactobacillus?* Or streptococcus in the genus *Serratia?*

4. Copy from any advanced text (such as Park and Williams' *Pathogenic Micro-organisms* or from Bergey's Manual), a complete description of 2 or 3 organisms which are given by name only at the extreme right margin of this key. Withholding the names themselves, exchange such descriptions with your classmates and endeavor to arrive at the correct genus in this key.*

* The old groups *Bacillus* and *Bacterium* being too large and unwieldy, have been subdivided into *Clostridium, Pasteurella,* etc. In the older texts most of these organisms are therefore still indexed under *Bacterium* and *Bacillus.*

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* To use this Index most efficiently, the following notes are needed:

1. Glossary definitions are not indexed, being themselves alphabetically arranged.

2. Names of authors occurring in the bibliographies (chapter endings, etc.), are not indexed.

3. Organisms have been listed under the related diseases, etc., and, contrary to custom, under *specific* rather than *generic* names. The recent changes in nomenclature, *e.g.*, *Bacillus* or *Bacterium coli* to *Escherishia coli*, will illustrate the difficulty beginners might find in a book with so little emphasis on morphology.

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